ΕΘΝΙΚΟ ΚΑΙ ΚΑΠΟΔΙΣΤΡΙΑΚΟ ΠΑΝΕΠΙΣΤΗΜΙΟ ΑΘΗΝΩΝ

ΙΑΤΡΙΚΗ ΣΧΟΛΗ

ΘΕΡΑΠΕΥΤΙΚΗ ΚΛΙΝΙΚΗ

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Προοπτική μελέτη παραγόντων κινδύνου και των μηχανισμών της πήξης στις πλασματοκυτταρικές δυσκρασίες

ΔΕΣΠΟΙΝΑ ΦΩΤΙΟΥ

ΙΑΤΡΟΣ

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Prospective study of thrombotic risk factors and coagulation mechanisms in plasma cell dyscrasias

DESPINA FOTIOU

DOCTOR

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Αφιερωμένο

Στον πατέρα μου, την αδερφή μου Ειρήνη και την μητέρα μου

Ευχαριστίες

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INDEX

B. THESIS

ΤΡΙΜΕΛΗΣ ΣΥΜΒΟΥΛΕΥΤΙΚΗ ΕΠΙΤΡΟΠΗ

ΕΥΣΤΑΘΙΟΣ ΚΑΣΤΡΙΤΗΣ (ΕΠΙΒΛΕΠΟΝ ΜΕΛΟΣ)

ΑΝ. ΚΑΘΗΓΗΤΗΣ, ΘΕΡΑΠΕΥΤΙΚΗ ΚΛΙΝΙΚΗ, Γ.Ν "ΑΛΕΞΑΝΔΡΑ", ΕΚΠΑ

ΜΕΛΕΤΙΟΣ ΑΘΑΝΑΣΙΟΣ ΔΗΜΟΠΟΥΛΟΣ

ΚΑΘΗΓΗΤΗΣ, ΔΙΕΥΘΥΝΤΗΣ ΘΕΡΑΠΕΥΤΙΚΗΣ ΚΛΙΝΙΚΗΣ, Γ.Ν "ΑΛΕΞΑΝΔΡΑ", ΕΚΠΑ

ΜΙΧΑΗΛ ΒΟΥΛΓΑΡΕΛΗΣ

ΚΑΘΗΓΗΤΗΣ, ΛΑΙΚΟ ΝΟΣΟΚΟΜΕΙΟ, ΕΚΠΑ

ΕΠΤΑΜΕΛΗΣ ΕΠΙΤΡΟΠΗ

ΕΥΣΤΑΘΙΟΣ ΚΑΣΤΡΙΤΗΣ

ΑΝ. ΚΑΘΗΓΗΤΗΣ, ΘΕΡΑΠΕΥΤΙΚΗ ΚΛΙΝΙΚΗ, Γ.Ν "ΑΛΕΞΑΝΔΡΑ", ΕΚΠΑ

ΜΕΛΕΤΙΟΣ ΑΘΑΝΑΣΙΟΣ ΔΗΜΟΠΟΥΛΟΣ

ΚΑΘΗΓΗΤΗΣ, ΔΙΕΥΘΥΝΤΗΣ ΘΕΡΑΠΕΥΤΙΚΗΣ ΚΛΙΝΙΚΗΣ, Γ.Ν "ΑΛΕΞΑΝΔΡΑ", ΕΚΠΑ

ΜΙΧΑΗΛ ΒΟΥΛΓΑΡΕΛΗΣ

ΚΑΘΗΓΗΤΗΣ, ΛΑΙΚΟ ΝΟΣΟΚΟΜΕΙΟ

ΕΥΑΓΓΕΛΟΣ ΤΕΡΠΟΣ

ΚΑΘΗΓΗΤΗΣ, ΘΕΡΑΠΕΥΤΙΚΗ ΚΛΙΝΙΚΗ, Γ.Ν "ΑΛΕΞΑΝΔΡΑ", ΕΚΠΑ

ΦΛΩΡΑ ΖΑΓΟΥΡΗ

ΑΝ. ΚΑΘΗΓΗΤΡΙΑ, ΘΕΡΑΠΕΥΤΙΚΗ ΚΛΙΝΙΚΗ, Γ.Ν "ΑΛΕΞΑΝΔΡΑ", ΕΚΠΑ

ΜΑΡΙΑ ΓΑΒΡΙΑΤΟΠΟΥΛΟΥ

ΕΠΙΚΟΥΡΗ ΚΑΘΗΓΗΤΡΙΑ, ΘΕΡΑΠΕΥΤΙΚΗ ΚΛΙΝΙΚΗ, Γ.Ν "ΑΛΕΞΑΝΔΡΑ", ΕΚΠΑ

ΜΑΡΙΑΝΝΑ ΠΟΛΙΤΟΥ

ΑΝ. ΚΑΘΗΓΗΤΡΙΑ, ΑΡΕΤΑΙΕΙΟ ΝΟΣΟΚΟΜΕΙΟ, ΕΚΠΑ

ΠΕΡΙΛΗΨΗ

Η φλεβική θρομβοεμβολή (ΦΘΕ) παραμένει μια από τις πιο συχνές επιπλοκές σε ασθενείς με Πολλαπλούν Μυέλωμα (ΠΜ) και περίπου 10% των ασθενών με νέα διάγνωση ΠΜ (ΝΔΠΜ) θα εκδηλώσουν ΦΘΕ κατά τη διάρκεια της νόσου τους. Τα περισσότερα συμβάματα λαμβάνουν χώρα τους πρώτους μήνες μετά τη διάγνωση της νόσου και την έναρξη της θεραπείας, με την επίπτωση αυτών να είναι υψηλότερη σε ασθενείς με νέα διάγνωση έναντι ασθενών με υποτροπιάζουσα ή ανθεκτική νόσο. Η πιο συχνή εντόπιση είναι η εν τω βάθει ΦΘΕ. Τα περισσότερα δεδομένα από κλινικές μελέτες υποστηρίζουν ότι η εκδήλωση ΦΘΕ σχετίζεται με μικρότερα ποσοστά συνολικής επιβίωσης. Συγκριτικά με το μέσο κίνδυνο ΦΘΕ στο γενικό πληθυσμό, τα ποσοστά ΦΘΕ είναι επίσης υψηλότερα στα προσυμπτωματικά στάδια της νόσου, δηλαδή σε ασθενείς με ασυμπτωματικό ΠΜ (ΑΠΜ) και μονοκλωνική γαμμαπάθεια αδιευκρίνιστης σημασίας (ΜΓΑΣ) υποδεικνύοντας έτσι ένα πιθανό αιτιοπαθογενετικό ρόλο του μονοκλωνικού πλασματοκυττάρου στην υπερπηκτικότητα που παρατηρείται σε αυτή την κατηγορία νοσημάτων.

Η υπερπηκτικότητα που παρατηρείται στο ΠΜ είναι πολυπαραγοντική και οι παράγοντες κινδύνου εκδήλωσης ΦΘΕ διαχωρίζονται σε κλινικούς παράγοντες κινδύνου σχετιζόμενους με τον ασθενή, παράγοντες κινδύνου που σχετίζονται με το υποκείμενο νόσημα καθώς και παράγοντες κίνδυνου σχετιζόμενους με το είδος της χορηγούμενης θεραπείας. Το είδος του θεραπευτικού σχήματος παίζει καθοριστικό ρόλο στην εκτίμηση του κινδύνου ΦΘΕ καθώς το ποσοστό ΦΘΕ 1-2% που σχετίζεται με κλασσικά σχήματα όπως μελφαλάνη και πρεδνιζολόνη αυξάνεται στο 26% όταν χρησιμοποιούνται ανοσοτροποποιητικοί παράγοντες σε συνδυασμό με υψηλές δόσεις δεξαμεθαζόνης ή σχήματα με πολλαπλούς χημειοθεραπευτικούς παράγοντες. Το προφίλ πήξης του ασθενή με ΠΜ δεν έχει κατανοηθεί μέχρι σήμερα επαρκώς. Πολλές ομάδες έχουν στρέψει τις ερευνητικές τους προσπάθειες στη διερεύνηση και κατανόηση της παθολογίας της πήξης που προκύπτει από την αλληλεπίδραση του μονοκλωνικού πλασματοκυττάρου, του μικροπεριβάλλοντος του μυελού των οστών και των παραγόντων της πήξης.

Η Διεθνής Ομάδα Εργασίας του Μυελώματος (International Myeloma Working Group IMWG) το 2014 και το Ευρωπαϊκό Δίκτυο Μυελώματος (European Myeloma Network) το 2015 δημοσίευσαν αλγόριθμο για την κατάλληλη διαστρωμάτωση του κινδύνου ΦΘΕ σε ασθενείς με ΠΜ που λαμβάνουν ανοσοτροποποιητικό παράγοντα (Immunomoduatory

agent – ImiD) και την προφυλακτική αγωγή που θα πρέπει να ακολουθείται με βάση αυτή τη διαστρωμάτωση. Ο αλγόριθμος βασίζεται κυρίως στην γνώμη των ειδικών και λιγότερο σε ισχυρά κλινικά δεδομένα καθώς αυτά είναι πολύ περιορισμένα. Η σύσταση είναι να χρησιμοποιείται ασπιρίνη χαμηλής δοσολογίας σε ασθενείς με κανένα ή έναν παράγοντα κινδύνου ΦΘΕ που λαμβάνουν IMiD και ηπαρίνη χαμηλού μοριακού βάρους σε προφυλακτική δόση ή κουμαρινικά σε θεραπευτική δόση σε ασθενείς που λαμβάνουν IMiD και έχουν περισσότερους του ενός παράγοντες κινδύνου ΦΘΕ. Η σύσταση είναι να χορηγείται η αγωγή θρομβοπροφύλαξης για 4-6 μήνες. Δεν υπάρχει δημοσιευμένος αλγόριθμος για ασθενείς που λαμβάνουν αγωγή που δεν περιλαμβάνει ανοσοτροποιητικό παράγοντα. Πρόσφατα δεδομένα υποστηρίζουν πως η εφαρμογή του προτεινόμενου αλγόριθμου στην καθημερινή κλινική πρακτική είναι ασυνεπής. Το ποσοστό υπολειπόμενων συμβαμάτων ΦΘΕ παρά την εφαρμογή των οδηγιών παραμένει υψηλό και υποδηλώνει πως ο προτεινόμενος αλγόριθμος και η διαστρωμάτωση κινδύνου στην οποία βασίζεται έχουν ατέλειες.

Η σχετιζόμενη με την κακοήθεια θρόμβωση είναι ένα ερευνητικό πεδίο με πολύ μεγάλο ενδιαφέρον και έχει επιτευχθεί σημαντική πρόοδος τα τελευταία χρόνια. Ένα από τα ορόσημα υπήρξε η ανάπτυξη και δημοσίευση του σκορ του Khorana το 2008 για την αξιολόγηση κινδύνου ΦΘΕ σε ασθενείς με συμπαγείς κακοήθειες. Έχει προταθεί από πολλούς ερευνητές ότι η ενσωμάτωση βιοδεικτών της υπερπηκτικότητας και ενεργοποίησης ενδοθηλίου στα μοντέλα αξιολόγησης κινδύνου ΦΘΕ μπορεί να αυξήσει την ευαισθησία τους και να βελτιώσει την απόδοσή τους. Ένας τέτοιος βιοδείκτης μπορεί να γενικευτεί για όλες τις κακοήθειες ή να αφορά σε μια συγκεκριμένη πάθηση αλλά για να αξιοποιηθεί θα πρέπει να μπορεί να μετρηθεί σε μη εξειδικευμένα εργαστήρια. Βελτιωμένες εκδόσεις του μοντέλου Khorana με καλύτερη προγνωστική αξία και ενισχυμένη απόδοση έχουν συμπεριλάβει και βιοδείκτες (Vienna CATS, Protecht score and 4TS-COMPASSE RAM). Η απόδοση των σκορ αυτών στο Μυέλωμα δεν είναι όμως ικανοποιητική. Δύο κλινικά σκορ παρουσιάστηκαν πρόσφατα για την εκτίμηση του κινδύνου ΦΘΕ σε ασθενείς με ΠΜ (IMPEDE and SAVED) αλλά αυτά δεν συμπεριλαμβάνουν βιοδείκτες υπερπηκτικότητας και αναπτύχθηκαν αναδρομικά. Υπάρχει επομένως ανάγκη για ένα αποτελεσματικό και προοπτικά επικυρωμένο μοντέλο αξιολόγησης κινδύνου το οποίο θα καλύπτει όλες τις πτυχές του προθρομβωτικού περιβάλλοντος που συναντάται

στους ασθενείς με ΠΜ. Υπάρχει επίσης έλλειψη δεδομένων όσον αφορά στην καταλληλότερη, ασφαλέστερη και πιο αποτελεσματική μέθοδο φαρμακευτικής θρομβοπροφύλαξης. Χρειάζονται δεδομένα από τυχαιοποιημένες κλινικές μελέτες που θα απαντήσουν ερωτήματα αναφορικά με την πλέον κατάλληλη μέθοδο θρομβοπροφύλαξης για τον εκάστοτε ασθενή με ΠΜ και το χρονικό διάστημα που θα πρέπει να χορηγείται. Ο ρόλος και η θέση των νεότερων από του στόματος αντιπηκτικών στην προφύλαξη και θεραπεία της σχετιζόμενης με κακοήθεια ΦΘΕ αποτελεί αντικείμενο μελέτης όλο και περισσότερων ερευνητικών ομάδων.

Η συγκεκριμένη διδακτορική διατριβή αποτελεί μέρος της συνεχιζόμενης προοπτικής κλινικής μελέτης ROADMAP-MM-CAT (PROspective Risk Assessment anD bioMArkers of hypercoagulability for the identification of patients with Multiply Myeloma at risk for Cancer-Associated Thrombosis, ClinicalTrials.gov identifier NCT03405571). Σκοπός της μελέτης είναι η διερεύνηση του υπερπηκτικού προφίλ (κυτταρικής υπερπηκτικότητας και υπερπηκτικότητας του πλάσματος) ασθενών με πλασματοκυτταρικές δυσκρασίες και συγκεκριμένα ασθενών με ΠΜ, ΑΣΠΜ και ΜΓΑΣ ώστε να καθοριστούν τα άτομα υψηλού κινδύνου εκδήλωσης ΦΘΕ που χρήζουν θρομβοπροφύλαξης. Στόχος της μελέτης είναι επίσης ο καθορισμός βιοδεικτών της πήξης και παραμέτρων που σχετίζονται με το ΠΜ καθώς και άλλων κλινικών παραγόντων κινδύνου ΦΘΕ που μπορούν να συνδυαστούν σε ένα μοντέλο αξιολόγησης κινδύνου ΦΘΕ σε ασθενείς με ΠΜ. Το πρωτογενές καταληκτικό σημείο είναι η εκδήλωση συμπτωματικής ΦΘΕ και δευτερογενή καταληκτικά σημεία συνιστούν η θνητότητα, το μείζον αιμορραγικό συμβάν, η ανταπόκριση της νόσου στην θεραπεία, η πρόοδος νόσου και η θνησιμότητα κατά τη διάρκεια της παρακολούθησης. Εντάχθηκαν στην μελέτη ασθενείς με νέα διάγνωση ΠΜ, ΑΠΜ και ΜΓΑΣ που παρακολουθούνται στο τμήμα πλασματοκυτταρικών δυσκρασιών της Θεραπευτικής Κλινικής του ΓΝΑ Αλεξάνδρα οι οποίοι είναι άνω των 18 ετών και δεν λαμβάνουν αντιπηκτική αγωγή. Η παρακολούθηση των ασθενών, η καταγραφή των κλινικών δεδομένων και η λήψη αιματολογικού ελέγχου έγινε κατά την ένταξη στην μελέτη καθώς και στους 3, 6 και 12 μήνες. Μελετήθηκε ένα εκτενές πάνελ βιοδεικτών κυτταρικής υπερπηκτικότητας και πηκτικότητας του πλάσματος. Οι μετρήσεις έγιναν σε κεντρικό εργαστήριο στο Thrombosis Center, Service d'Hématologie Biologique, Tenon University Hospital, Paris. Συνολικά 480 ασθενείς με νέα διάγνωση ΠΜ, ΑΠΜ και ΜΓΑΣ έχουν ενταχθεί

στην μελέτη από τον Ιούνιο του 2014 μέχρι τον Ιούνιο του 2018. Η διαστρωμάτωση κινδύνου και η χορήγηση αντιπηκτικής αγωγής για προφύλαξη ΦΘΕ έγιναν με βάση τα κριτήρια της IMWG.

Η μέτρηση των βιοδεικτών της πήξης και η ανάλυση των δεδομένων έχει γίνει σε συνολικά 144 ασθενείς με ΠΜ, 80 ασθενείς με ΑΠΜ και 54 ασθενείς με ΜΓΑΣ. Το ποσοστό ΦΘΕ κατά τη διάρκεια των 12 μηνών παρακολούθησης των 144 ασθενών με ΠΜ ήταν 10.4%. Δεν παρατηρήθηκαν ΦΘΕ στους ασθενείς με ΑΠΜ και ΜΓΑΣ. Το μεγαλύτερο ποσοστό των συμβαμάτων έλαβε χώρα κατά τους 3 πρώτους μήνες από την έναρξη της θεραπείας και η πιο συχνή εντόπιση ήταν η εν τω βάθει φλεβοθρόμβωση. Δεν υπήρχε στατιστικά σημαντική συσχέτιση μεταξύ του είδους της θεραπείας (βασισμένη σε ανοσοτροποποιητικό παράγοντα ή μη) αναφορικά με τα συμβάματα ΦΘΕ. Ο μικρός αριθμός των περιπτώσεων ΦΘΕ δεν ήταν ικανός για να εντοπιστεί τέτοια συσχέτιση. Δεν ανεδείχθη επίσης συσχέτιση μεταξύ της ΦΘΕ και του είδους της αντιπηκτικής αγωγής που λάμβαναν οι ασθενείς. Με βάση τα αποτελέσματα της μελέτης και τους βιολογικούς δείκτες που μελετήθηκαν οι ασθενείς με νέα διάγνωση ΠΜ έχουν προφίλ κυτταρικής υπερπηκτικότητας και υπερπηκτικότητας του πλάσματος. Ο χρόνος πήξης εξαρτώμενος από τα προπηκτικά φωσφολιπίδια (Procoagulant-PPL) ήταν συντομότερος, τα επίπεδα P-σελεκτίνης μικρότερα και η παραγωγή θρομβίνης συνολικά κατεσταλμένη συγκριτικά με τους υγιείς μάρτυρες. Οι ασθενείς με ΜΓΑΣ και ΑΠΜ έχουν επίσης προφίλ κυτταρικής υπερπηκτικότητας και υπερπηκτικότητας του πλάσματος το οποίο είναι παρόμοιο αλλά όχι ταυτόσημο με αυτό των ασθενών με ΠΜ. Μεταβαίνοντας από τους ασθενείς με ΜΓΑΣ στους ασθενείς με ΑΠΜ και στους ασθενείς με ΠΜ τα επίπεδα Δ-διμερών και του μονομερούς του ινώδους αυξάνονται και η παραγωγή θρομβίνης καταστέλλεται περαιτέρω.

Από τις κλινικές παραμέτρους που μελετήθηκαν μόνο η ενεργή πνευμονική νόσος σαν έμμεσος δείκτης χρόνιας φλεγμονής φάνηκε να σχετίζεται στατιστικά σημαντικά με την εμφάνιση ΦΘΕ. Παρατηρήθηκε επίσης μια αντιστρόφως ανάλογη συσχέτιση μεταξύ της μονοκλωνικής παραπρωτεΐνης (Mpeak) και της εμφάνισης ΦΘΕ, η οποία δεν ήταν αναμενόμενη. Η σημασία του ευρήματος αυτού μένει να εκτιμηθεί σε μελλοντικές αναλύσεις μεγαλύτερων δειγμάτων ασθενών και συμβαμάτων ΦΘΕ. Στους 3 μήνες μετά την έναρξη θεραπείας το παθολογικό βιολογικό προφίλ των ασθενών με ΠΜ ως επί το

πλείστον δεν είχε αναστραφεί. Παρατηρήθηκε όμως μείωση του ιστικού παράγοντα (tissue factor), του αναστολέα του μονοπατιού του ιστικού παράγοντα, του παράγοντα FVIII, των Δ-διμερών και αύξηση του παράγοντα FV.

Από τους βιοδείκτες της πήξης που μελετήθηκαν, ο μεγαλύτερος χρόνος πήξης εξαρτώμενος από τα προπηκτικά φωσφολιπίδια (Procoag-PPL), η μικρότερη παραγωγή θρομβίνης (μικρότερο Endogenous thrombin potential ETP) και τα υψηλότερα επίπεδα αναστολέα του μονοπατιού του ιστικού παράγοντα (Tissue factor pathway inhibitor - TFPI) σχετίζονται με την εμφάνιση ΦΘΕ. Στην πολυπαραγοντική ανάλυση φάνηκε πως το Procoag-PPL και ETP είναι ανεξάρτητοι παράγοντες κινδύνου για την εμφάνιση ΦΘΕ. Με βάση το μοντέλο της πολυπαραγοντικής ανάλυσης δημιουργήθηκε ένα σκορ. Δίνεται 1 βαθμός για Procoag-PPL® ≥47 sec και 1 βαθμός για ETP <1087 nMxmin, ή 0 βαθμοί για Procoag-PPL® <47 sec και ETP ≥1087 nMxmin αντίστοιχα. Με βάση το σκορ αυτό οι ασθενείς κατηγοριοποιούνται σε χαμηλού/ενδιάμεσου κινδύνου και σε υψηλού κινδύνου για εμφάνιση ΦΘΕ Το ποσοστό ΦΘΕ στην ομάδα χαμηλού/ενδιάμεσου κινδύνου ήταν 5% και στην ομάδα υψηλού κινδύνου 17.5%. Η ευαισθησία και η ειδικότητα του σκορ ήταν 71.4% και 61.8% αντίστοιχα.

Με βάση τα δεδομένα που παρουσιάζονται το Procoag-PPL® και το ETP μπορούν να ενταχθούν προοπτικά σε ένα μοντέλο αξιολόγησης κινδύνου ΦΘΕ στους ασθενείς με ΠΜ σε συνδυασμό με κλινικούς παράγοντες κινδύνου και παράγοντες κινδύνου σχετιζόμενους με το νόσημα. Ένα τέτοιο σκορ/μοντέλο αξιολόγησης κινδύνου αναμένεται να βελτιστοποιήσει την διαστρωμάτωση κινδύνου ΦΘΕ σε ασθενείς με ΠΜ και να επιτρέψει την αποτελεσματική και ασφαλή επιλογή εκείνων των ασθενών που χρήζουν θρομβοπροφύλαξης. Η ένταξη ενός τέτοιου μοντέλου στις κατευθυντήριες οδηγίες και στις κλινικές μελέτες που αξιολογούν την αποτελεσματικότητα και την ασφάλεια των νεότερων αντιπηκτικών θα οδηγήσει σε σημαντική μείωση της επίπτωσης αυτής της σημαντικής επιπλοκής στους ασθενείς με ΠΜ. Η ανάπτυξη ενός τέτοιου μοντέλου είναι ανάμεσα στους βασικούς στόχους της τρέχουσας μελέτης ROADMAP-MM-CAT.

ABSTRACT

Venous thromboembolism (VTE) remains one of the most common complications in patients with multiple myeloma (MM) and approximately 10% of patients with newly diagnosed MM (NDMM) will develop VTE during their disease course. According to an older report on the incidence of thrombosis in patients with haematological malignancies, the crude rate of deep vein thrombosis (DVT) during an 8 year follow up of more than 6000 MM patients was 8%. Most events occur within the first months following diagnosis and treatment initiation and the rates are higher in newly diagnosed compared to relapsed or refractory patients. The most frequent localization is DVT. VTE events are associated with higher morbidity and most data support an inferior overall survival in MM patients with VTE. Compared to the standard VTE risk in the population, rates are higher also in pre-symptomatic stages of the disease like monoclonal gammopathy of undetermined significance (MGUS) pointing to a possible prothrombotic role or nature of the monoclonal plasma cell.

The thrombogenicity in myeloma is multifactorial and risk factors associated with VTE occurrence in MM patients have long been distinguished in patient-related clinical risk factors, myeloma-related risk factors and finally risk linked to the type of anti-myeloma treatment administered. The treatment regimen of choice is one of the major determinants of VTE risk; the standard 1-2% VTE rates associated with conventional regimens such as melphalan and prednisone increase to about 4% with immunomodulatory drug monotherapy and up to 26% in some reports when immunomodulatory agents (IMiDs) are combined with high dose corticosteroids or multi-agent chemotherapy. The coagulation profile of the MM patient is still poorly understood. Many groups have turned their research efforts towards delineating the coagulation abnormalities that result from the crosstalk between the monoclonal plasma cell, the bone marrow microenvironment and components of coagulation.

The International Myeloma Working Group (IMWG) 2014 statement and the European Myeloma Network Guidelines in 2015 proposed a risk stratification algorithm and relevant thromboprophylaxis guidelines for MM patients who receive IMiDs. The algorithm is based on limited clinical trial data and mostly on expert opinion. The recommendation is to use

low dose aspirin for Newly diagnosed MM patients on IMiDs when one or none risk factors are present and prophylactic LMWH or dose-adjusted therapeutic warfarin when 2 or more risk factors for VTE are present for a duration of 4-6 months. There are no guidelines available for MM patients who are not on IMiD containing regimens. Recent reports demonstrate that the incorporation of the above recommendations in every day clinical practice is to say the least inconsistent. The rate of residual VTE rates remains high pointing to the suboptimal value and performance of the current risk stratification tools for VTE in MM patients.

Cancer associated thrombosis (CAT) is an area of very active research efforts and considerable progress has taken place since the introduction of the Khorana risk score in 2008 for VTE risk in solid tumors. Many groups have recommended that the incorporation of biomarkers of blood hypercoagulability and endothelial cell activation can increase the sensitivity of Risk assessment models (RAMs) for VTE risk identification. A biomarker can be either generic for all malignancies or disease specific but to be useful is should be measured with tools that are readily available in non-specialized laboratories. Improved versions of the Khorana score in terms of predictive value and enhanced performance with the incorporation of coagulation markers have been introduced (Vienna CATS, Protecht score and 4TS-COMPASSE RAM). The performance of these scores in MM is poor. Two clinical scores for VTE assessment have been presented recently (IMPEDE and SAVED) but these do not include biomarkers of coagulation, and were developed retrospectively. An effective and prospectively validated Risk assessment model (RAM) is therefore needed that can capture all aspects of the pro-thrombotic environment that exists in MM patients. In addition to the need for an MM-specific RAM there is a paucity of data regarding the most appropriate, safe and effective agent for thromboprophylaxis. Data from Randomized clinical trial is needed to answer questions such as what is the right agent and for which MM patient and what amount of time? The role of Direct oral anticoagulants (DOACs) or Non-vitamin K oral anticoagulants (NOACs) is being increasingly investigated in CAT treatment and prevention.

The current thesis is part of the ongoing prospective, investigator initiated ROADMAP-MM-CAT (PROspective Risk Assessment anD bioMArkers of hypercoagulability for the identification of patients with Multiply Myeloma at risk for Cancer-Associated Thrombosis,

ClinicalTrials.gov identifier NCT03405571) trial. The aim of the trial is to explore the coagulation profile (plasma and cellular hypercoagulability) of patients with plasma cell dyscrasias and in particular Multiple Myeloma (MM), Smoldering Multiple myeloma (SMM) and monoclonal gammopathy of underdetermined significance (MGUS) and to determine which MM patients are at high risk of VTE and therefore eligible for thromboprophylaxis for VTE. We also aimed to identify in NDMM patients relevant biomarkers of hypercoagulability, variables related with MM and clinical predictors of VTE risk that could be combined in a RAM for CAT in MM patients. The primary end point was the occurrence of symptomatic venous thromboembolism and secondary end-points mortality, major bleeding, disease response to treatment and progression and morbidity during follow-up.

Patients with a new diagnosis of MM, SMM and MGUS followed up in the department of Clinical Therapeutics, Plasma cell Dyscrasia Unit, General Alexandra Hospital who were >18 years of age and not on anticoagulation treatment were enrolled in the study. A clinical research form was completed and blood tests were obtained at enrollment (baseline), 3 months, 6 months and 12 months. An extensive panel of coagulation biomarkers were measured by centralization of blood samples to the core laboratory at Thrombosis Center, Service d'Hématologie Biologique, Tenon University Hospital, Paris. A total of 480 patients with a new diagnosis of MM, SMM and MGUs were enrolled in the study from June 2014 up to June 2019. Risk stratification was performed according to the IMWG guidelines and thromboprophylaxis was initiated accordingly. Data and sample analysis has been performed in a total of 144 MM patients, 80 SMM patients and 54 MGUS patients who were enrolled from June 2014 to June 2017. At 12month follow-up of 144 MM patients cumulative VTE rate was 10.4%. No events were observed in the SMM and MGUS groups. Most events occurred during the first 3 months since treatment initiation and the most common localization was Deep vein thrombosis (DVT). The VTE rate was not significantly associated with the type of treatment patient's received (IMiD-based versus non-IMiD based) as the study was not powered to detect such differences. In addition there was no association between VTE occurrence and the type of thromboprophylaxis patients received. NDMM patients showed biological signs of cellular and plasma hypercoagulability and endothelial cell activation. Procoagulant phospholipid clotting time (Procoagulant-PPL) was shorter, P-selectin levels lower and thrombin generation attenuated overall compared to

healthy subjects. Patients with SMM and MGUS also showed signs of cellular and plasma hypercoagulability with a similar but not identical profile to MM patients. Along the MGUs-SMM- MM continuum there was an increase in D-dimer levels, fibrin monomer levels and longer thrombin generation lagtime.

Among clinical parameters studied only pulmonary disease as a marker of chronic inflammation was significantly associated with VTE occurrence. Surprisingly monoclonal M-protein was inversely associated with VTE occurrence. The significance of this finding needs to be evaluated in future larger cohorts. Following 3 months of treatment most of the coagulation abnormalities detected at baseline were not reversed. The changes that were detected include a reduction in tissue factor (TF), tissue factor pathway inhibitor (TFPI), FVII and D-dimer and an increase in FV levels. Among coagulation biomarkers, longer Procoag-PPL®, lower Endogenous thrombin potential (ETP) and higher levels of Tissue factor pathway inhibitor (TFPI) were associated with VTE occurrence. Multivariate analysis showed that Procoag-PPL® and ETP were independent risk factors for VTE. A multivariate logistic regression model was developed and a score formulated. In the score 1 point was given for Procoag-PPL® ≥47, and 1 for ETP <1087 nMxmin), or 0 for Procoag-PPL® <47 sec, and ETP ≥1087 nMxmin respectively. The patients were stratified in two groups; high and intermediate/low risk group. The VTE rate was 5% in the intermediate/low risk group and 17.5% in the high risk group. The sensitivity and the specificity of the score was 71.4% and 61.8%, respectively.

Based on our data Procoag-PPL® and ETP can be prospectively incorporated into a RAM for VTE in MM in combination with clinical and disease risk factors. Such a score would optimize risk stratification of patients with MM and would allow selection of patients who are candidates for thromboprophylaxis in an efficient and safe manner. The incorporation of such a model in published guidelines by expert groups and its use in clinical trials that assess the safety and efficacy of newer anticoagulants will lead to a considerable reduction of the VTE rate in the MM population. The development of such a score is part of the ongoing ROADMAP-MM-CAT clinical trial.

GENERAL PART

Plasma cell dyscrasias:

Plasma cells are terminally differentiated, non-dividing immune cells that arise from B cells. Their primary function is to secrete antibodies with different heavy chain (IgM, IgG, IgA, IgD, and IgE) and a light chain (kappa and lambda) characteristics. Like all cells there are susceptible to transformation and clonal populations can develop. An early finding in plasma cell dyscrasias (PCD) with the exception of few non-secreting subtypes, is the presence of a monoclonal heavy and light chain antibody, referred to as paraprotein or monoclonal M-protein. The heterogenous group of plasma cell dyscrasias includes: Monoclonal gammopathy of undetermined significance (MGUS), Asymptomatic/ Smoldering multiple myeloma (SMM), Multiple myeloma (MM), Solitary plasmacytoma, Waldenstrom's Macroglobulinemia (WM), AL amyloidosis, Monoclonal immunoglobulin deposition disease, Light chain deposition disease, POEMS syndrome, Primary plasma cell leukemia (PPCL). Definitions for plasma cell dyscrasias are presented in Table 1. (1)

The current thesis has focused on the coagulation abnormalities and thrombosis mechanisms in MM, SMM and MGUS.

IgM MGUS	All three criteria must be met: • Serum monoclonal protein <3mg/dL and abnormal FLC ratio (<0.26 or >1.65) and increased level of involved light chain (increased κ FLC in patients with FLC ratio >1.65 and increased λ FLC in patients with FLC ratio <0.26) and urinary monoclonal protein <500 mg per 24 h • Bone marrow lymphoplasmacytic infiltration <10% • No evidence of end organ damage be attributed to the underlying lympho- plasmacytic proliferative disorder
Smoldering Myeloma	• Bone marrow clonal plasma cell infiltration ≥10% but <60% • Or ≥3 g/dL of monoclonal protein (Mprotein) in serum (or ≥500 mg/24 h in urine) or both • involved to uninvolved serum free light chain ratio of < 100 absence of any end-organ damage
Solitary plasmacytoma (2, 3)	• Biopsy-proven solitary lesion of bone or soft tissue with evidence of clonal plasma cells • Normal bone marrow with no evidence of clonal plasma cells • Normal skeletal survey and MRI (or CT) of spine and pelvis (except for the primary solitary lesion) Absence of end-organ damage such as hypercalcaemia, renal insufficiency, anaemia, or bone lesions (CRAB) that can be attributed to a lymphoplasma cell proliferative disorder
Solitary plasmacytoma with minimal marrow involvement	• Biopsy-proven solitary lesion of bone or soft tissue with evidence of clonal plasma cells • Clonal bone marrow plasma cells <10% • Normal skeletal survey and MRI (or CT) of spine and pelvis (except for the primary solitary lesion) • Absence of end-organ damage such as hypercalcaemia, renal insufficiency, anaemia, or bone lesions (CRAB) that can be attributed to a lymphoplasma cell proliferative disorder
Myeloma	• Clonal BM plasma cells ≥10% or biopsy proven plasmacytoma • Presence of CRAB features that can be attributed to a lymphoplasma cell proliferative disorder • Or Myeloma defining events: clonal bone marrow plasma cell percentage (BMPC) of ≥60%, an involved to uninvolved serum free light chain ratio of ≥ 100, two or more focal lesions on MRI) Non secretory Myeloma: all of the above but negative serum and urine electrophoresis and immunofixation and normal free light chain assay Oligo-secretory Myeloma: all of the above but serum monoclonal protein <1g/dl and urine monoclonal protein <200mg/day

POEMS syndrome (4)	• Polyneuropathy • Monoclonal plasma cell proliferative disorder (almost always λ) Any one of the following three other major criteria: • Sclerotic bone lesions • Castleman's disease • Elevated levels of VEGFA Any one of the following six minor criteria: • Organomegaly (splenomegaly, hepatomegaly, or lymphadenopathy) • Extravascular volume overload (edema, pleural effusion, or ascites) • Endocrinopathy (adrenal, thyroid, pituitary, gonadal, parathyroid, pancreatic) • Skin changes (hyperpigmentation, hypertrichosis, glomeruloid haemangiomata, plethora, acrocyanosis, flushing, white nails) • Papilledema • Thrombocytosis/polycythemia
Systemic AL amyloidosis (5)	• Presence of an amyloid-related systemic syndrome (eg, renal, liver, heart, gastrointestinal tract, or peripheral nerve involvement) • Positive amyloid staining by Congo red in any tissue (eg, fat aspirate, bone marrow, or organ biopsy) • Evidence that amyloid is light-chain-related established by direct examination of the amyloid using mass spectrometry-based proteomic analysis, or immunonoelectron microscopy, and • Evidence of a monoclonal plasma cell proliferative disorder (serum or urine monoclonal protein, abnormal free light-chain ratio, or clonal plasma cells in the bone marrow)
Plasma cell leukemia	Presence of ≥ 20% circulating plasma cells on conventional white blood cell differential count and an absolute plasma cell count of ≥ 2×10^9/L in the peripheral blood (definition considered too stringent and recommendations for using lower thresholds are made)
Waldenstrom's macroglobulinemia	• ≥10% Lymphoplasmacytic infiltration in the bone marrow or lymphatic tissue • Monoclonal immunoglobulin M protein in the serum Criteria for treatment initiation: cytopenias, constitutional symptoms, lymphadenopathy or organomegaly, neuropathy, amyloidosis, cryoglobulinemia, hemolytic anemia, cold agglutinin disease

Table 1: Table 1: Monoclonal gammopathies or plasma cell dyscrasias and International Myeloma Working Group definitions. MGUS: monoclonal gammopathy of undetermined significance; FLC: free light chain

1. Multiple Myeloma:

1.1 Definition and Characteristics:

Multiple myeloma (MM) is a hematological malignancy of terminally differentiated plasma cells which reside usually in the bone marrow but can also be found in other extramedullary sites and in the peripheral blood. Most commonly there is secretion of a monoclonal immunoglobulin protein (M protein) by the abnormal plasma cells which can be detected and measured in the peripheral blood or urine but in 15-20% of patients the plasma cells secrete only free light chains and in <3% no monoclonal protein is secreted.(6) Clinical manifestations are the end result of the damage caused by the monoclonal protein, the plasma cell itself or cytokines, what is collectively known as CRAB features: C: hypercalcemia, R: renal insufficiency, A: anemia, B: bone disease. (1) The entity belongs to a group of disorders referred to as plasma cell dyscrasias or monoclonal gammopathies. Among them monoclonal gammopathy of undetermined significance (MGUS) is the most common. It is characterized by monoclonal plasma cells in the bone marrow and secretion of monoclonal protein but is asymptomatic. MGUS may precede the development of MM often with an identified intervening stage referred to as shouldering multiple myeloma or asymptomatic multiple myeloma (SMM). (7) The risk of progression to myeloma or related malignancy is about 1% per year for MGUS and high monoclonal protein level, high percentage of plasma cells in the bone marrow, IgA monoclonal protein and abnormal free light chain ratio .(8, 9) Landgren et al studied patients with MM and showed that almost 100% were found to have monoclonal peak 2,3,4,5,6,7, and over 8 years prior to their MM diagnosis.

1.2 Historical overview:

The first well documented case of patient with MM is that of Thomas Alexander McBean a 45 year old man who first presented with symptoms such as fatigue and bone pain in 1844 and eventually died in 1846 after having tried multiple therapies by his physician Thomas Watson. On autopsy his ribs were soft and brittle, a “gelatinoform substance of blood-red

color" was found in the bones and histologic examination of the bone marrow revealed round, one-half to twice as large as an average blood cell with one or 2 nuclei and a bright-colored nucleolus. (10) In addition the urine of Thomas Alexander McBean were sent to Henry Bence Jones at St. George's Hospital in 1845. Jones concluded that the protein in the urine was the "hydrated deutoxide of albumen" and that it played a major role in conditions of softening of the bone "mollities osseum". The term "plasma cell" was first used by Waldeyer in 1875 probably to describe mast cells, Ramon y Cajal was the first to correctly identify them and Marschalko in 1895 published the first accurate description. Arinkin introduced bone marrow aspiration which increased the recognition of the condition. In 1928 Geschickter and Copeland reported on 412 cases found in the literature of patients with pathological fractures, Bence Jones proteinuria, anemia and chronic renal disease. (10) Fleisher in 1880 was the first one to use the term "Bence Jones Protein" and Korngold and Lipari in 1959 identified the two different classes of Bence Jones proteins which were designated as a tribute to them kappa and lambda. (11)Later in 1962, Edelman and Gally showed that the amino acid composition of light chains prepared from an IgG monoclonal protein in the serum and the Bence Jones protein from the same patient's urine was identical. (12)

Perlzweig et al in 1928 were the first to demonstrate the presence of hypoproteinemia. Tiselius developed over the years the moving-boundary method of electrophoresis and together with Kabat in 1939 demonstrated the antibody activity in the gamma globulin fraction of the electrophoresis. The narrow-based peak in serum electrophoresis characteristic of MM was first recognized in 1939. Grabar and Williams described immunoelectrophoresis in 1953 and Wilson introduced immunofixation in 1964. (13) A critical milestone was also the distinction between monoclonal and polyclonal gammopathies presented by Jan Waldenström in the Harvey Lecture Series in 1961.

1.3 Epidemiology:

Multiple myeloma (MM) is a malignant plasma cell disorder that accounts for approximately 1.7% of all malignancies, 10% of all hematologic cancers and is the second

most common hematological malignancy. (14, 15) (16, 17) (18) It accounts for approximately 2% of all cancer deaths. (19) The median age of disease presentation is 71 and only 3% of patients will be less than 40 years old. MGUS, the premalignant indolent stage that often precedes MM is present in more than 3% of the population above the age of 50 and its cause is currently unknown. Myeloma and MGUS are twice as common in African Americans than in Caucasians, and even less common in Asian people and slightly more common in males than females (1.4:1.0). Worldwide in 2016 the age-standardized incidence rate (ASIR) of MM was 2.1 per 100 000 persons (95% UI, 1.8-2.3). The 3 world regions with the highest ASIR of MM were Australasia, North America, and Western Europe and Multiple myeloma caused 2.1 million DALYs globally in 2016. (20) In USA the average annual incidence increased from 1 per 100,000 person years between 1935-1944 to 2.9 cases per 100,000 person-years between 1945-1964. The age adjusted incidence was 4.6 per 100,000 person years between 1945 and 2001 in USA but no significant increase was observed over 3 year periods during this period. In Sweden the incidence also remained stable between 1950 and 2005 indicating that the increase in the incidence of MM is due to improved case ascertainment. (21, 22) Median overall survival for patients with MM has increased over the last decade to over 5 years owing to the introduction of novel agents, namely immunomodulatory agents (IMiDs), proteasome inhibitors (PIs) and monoclonal antibodies. (23, 24) It remains however an incurable disease.

1.1 Pathogenesis:

The underlying cause of MM is unknown. Environmental and occupational exposures have been studied by some groups but no direct link has ever been established. One study reported an increased incidence in individuals who received radiation exposure in Hiroshima and Nagasaki atomic bomb incidents compared to controls but a more recent analysis did not demonstrate a link between radiation exposure and MM. (25) Occupational exposures studied include pesticides, solvents, infectious agents, hair dyes, benzene and petroleum products but little evidence is available to support causal relationships. (21) The role of genetic factors is an area that has gained a lot of research interest recently as there are pointers to suggest underlying genetic factors but these have not been identified yet. The risk of MGUS in first-degree relatives of patients with MM is increased two times.(26) Numerous families with two or more first-degree relatives with multiple myeloma have

been reported. (27) Genetic loci and single nucleotide polymorphisms (SNPs) associated with increased risk of MM, inferior survival in diagnosed patients or drug-induced toxicities have been identified using genome-wide association studies (GWAS). (28, 29)

Plasma cells originate from hematopoietic stem cells which undergo differentiation in the bone marrow and secondary lymphoid organs to B cells and then to plasma cells. V(D)J rearrangement of immature B cells leads to the diverse primary immunoglobulins. B cells that have a IgG-IgL complex migrate to secondary lymphoma organs and undergo several processes (class-switch recombination, somatic hypermutation, affinity maturation) which results in antibody production. Class-switch recombination and somatic hypermutation required double-strand DNA breaks in the immunoglobulin but these DNA breaks can fuse with other breaks in the genome leading to aberrant DNA fusions and chromosomal translocations. Some of these translocations involve oncogenes that can give the cell an advantage leading to the development of pathological states such as MGUS, SMM and eventually MM. Chromosomal translocations are therefore considered a possible initiating event in some cases. Aneuploidy is another possibly contributing event. (21)

1.1.1 Genetic alterations:

Multiple genetic alterations have been proposed as driving events in myelomagenesis. (30, 31)

Chromosomal defects: An important class of primary events identified in MGUS, SMM and MM involve translocations in the gene encoding immunoglobulin heavy chains *(IGH*) and other partner genes, *NSD2, FGFR3* (fibroblast growth factor receptor 3 gene) and *CCND1* (encoding cyclin D1). Translocations occur mostly due to abnormal class-switch recombination but also V(D)J rearrangements. In 14% of MM patients translocation t(11;14) is found which results in increased expression of *CCND1* and consequently cyclin D1 which is important for cell cycle progression. Translocation t(4;14) is found in 11% of patients, leading to *NSD2* and often *FGFR3* overexpression. Other translocations that involve IGH include t(14;16) which is found in 3% of patients and involves *MAF,* t(14;20) which is found in 1.5% of patients and involves *MAFB* and t(6;14) which is found in <1% of patients and involves *CCND3*.

These translocations are also found in patients with MGUS; t(4;14) is seen in 1-3% of patients with MGUS and t(11;14) in 13%. (32) Hyperdiploidy is the most frequent type of aneuploidy in MM. In a recent series of MM patients characterized by SNP array, 35% had <46 chromosomes (hypodiploidy), 13% had 46 chromosomes (pseudodiploidy, 14% had 47-50 chromosomes (hyperdiploidy) and 38% had >50 chromosomes (large hyperdiploidy). (33) Among the trisomies identified, 3 and 5 are associated with good prognosis and 21 with adverse outcomes. In terms of ploidy, patients with hypodiploidy have the worst outcomes followed by those with pseudodiploidy and hyperdiploidy. Other chromosomal defects seen in MM include deletion of the long arm of chromosome 13 (del(13q)), loss of the short arm of chromosome 17 (del(17p)), gain of the long arm of chromosome 1 (gain(1q)) and loss of the short arm of chromosome 1 (del(1p)). The increased occurrence of del(17p) and translocation t(8;14) which links *IGH* on chromosome 14 with the *MYC* oncogene is association with progression to refractory disease and to plasma cell leukaemia. MYC has been found to be deregulated in up to 49% of patients with MM. (34)

1.1.2 Secondary mutations and clonal evolution:

NGS (next generation sequencing) has demonstrated the absence of a universal driver mutation in MM but the coexistence of subclones of malignant plasma cells with partially overlapping but unique mutations. (35) Among the most frequently occurring mutations are in *KRAS* (in 23% of patients), *NRAS* (20%), *FAM46C* (11%), *DIS3* (11%) and *TP53* (8%). In a subset of MM patients there is branching clonal development where one or more subclones appear and other disappear. This has consequences for treatments that target the mutated protein and a potential therapeutic strategy would be to use multiple targeted therapies to destroy all malignant subclones. In addition different biopsy sites within the same patient showed partially overlapping mutations and different mutations pointing to even higher degrees of genetic complexity. (36) Alterations in several cellular pathways can also be seen for example the nuclear-factor κB pathway.

Epigenetic alterations: Median global methylation is also variable in MM with both hyper and hypomethylation seen in some patients. DNA hypermethylation is increased in MM compared to MGUS and is seen at enhancer regions linked to reduced expression of the

relevant genes. The chromatin regulator bromodomain-containing protein 4 (BRD4) binds to enhancer sites associated with genes that have strong links with MM. Several miRNAs are also present at different levels in MM cells including miR-19a and miR-19b which contribute to Janus kinase-signal transducer and activator of transcription pathway.

1.1.3 Microenvironment

The interplay between the MM cells and the bone marrow microenvironment is crucial for myeloma development, treatment and progression. Cells types that compose the microenvironment and interact with plasma cell include haematopoietic cells (including B cells, T cells, natural killer cells, myeloid-derived suppressor cells and osteoclasts) and non-haematopoietic cells (including bone marrow stromal cells, osteoblasts and endothelial cells). Migration of plasma cells to the bone marrow involves expression of CXCR4 (chemokine receptor) on cells but endothelial cells also play a role via the secretion of extracellular cyclophilin A which binds to CD147. Initial clone formation in the bone marrow has been described as micrometastatic and the formation of additional localization of MM cells in the bone marrow as colonization. (37) The interaction of bone marrow stromal cells and MM cell leads to changes in the levels of receptor activator of nuclear factor-κB ligand (RANKL; also known as TNFSF11) and osteoprotegerin (also known as TNFRSF11B) which are involved in bone formation. There is increased activity of osteoclasts and reduced activity and number of osteoblasts via increased RANKL production and reduced levels of osteoprotegerin and the imbalance leads to bone destruction. (38) There is also a process termed cell adhesion-mediated drug resistance (CAMDR) which leads to treatment resistance via binding of MM cells to bone marrow stromal cells.(39) Exosomes may also have a role in the interplays that take place in the bone marrow microenvironment as some bone marrow stromal cells have lower content of some miRNAs which might affect tumor growth and development.

The mode of action of some important drug classes sheds light into two additional potential mechanisms that contribute to MM pathogenesis. Immunomodulatory agents (IMiDs) , thalidomide, lenalidomide and pomalidomide target cereblon which is part of an E3 ubiquitin ligase complex that causes ubiquitylation and degradation of several transcription factors (the DNA-binding protein Ikaros, the zinc finger protein Aiolos and casein kinase I

isoform-α (CK1a)). Ikaros and Aiolos expression causes increased levels of IRF4 which upregulates MYC and IRF4 inhibition is toxicity to multiple myeloma cell lines. Ikaros degradation also leads to increased IL-2 production in T cells. (40) Plasma cells including myeloma cells, have a physiological induction of the unfolded protein response to achieve antibody production and are therefore sensitive to therapies that increase stress on protein turnover such as proteasome inhibition. Overexpression of specific proteasome subunits and increased proteasome capacity has been linked to proteasome inhibitor (bortezomib) resistance. (41)

1.2 Clinical presentation

Patients with MM will usually present with fatigue and dyspnea due to anemia, bone pain due to bone disease or even neurological symptoms due to hypercalcemia and spinal cord compression. Anemia is seen in about 70% of patients and is attributed to bone marrow plasma cell infiltration but also to production of cytokines like IL-6, TFA-a, IL-1 which affect erythropoiesis and lead to decreased production of erythropoietin. MM patients with anemia response to external administration of erythropoietin. Renal impairment also contributes to anemia (42, 43). Bone marrow infiltration by plasma cells can also lead to neutropenia and thrombocytopenia.

Renal impairment is seen in about 20% of patients and underlying pathogenesis is multifactorial. In healthy individuals light chains are filtered at the glomerulus and reabsorbed a the proximal tubuli. In MM the capacity of reabsorption is exceeded and light-chains accumulate in the distal segment of the nephron where light chains combine with Tamm-Horsfall urinary glycoprotein and precipitate to form obstructing casts which cause renal impairment. The quantity of free light chain is not directly correlated to the occurrence of renal impairment, indicating that differences between light-chain species may contribute to causing this impairment. Cast nephropathy is the most common mechanisms of the underlying renal impairment seen in MM (44) (45). Other contributing factors are hypercalcemia, dehydration, hyperuricemia. Two other important mechanisms of renal

impairment in MM include light chain and heavy chain deposition disease and amyloid deposition in AL amyloidosis. In both entities renal impairment results in non-selective proteinuria. Acquired Fanconi syndrome has also been reported in rare cases. (46). About 10% of patients with MM will present with amyloidosis. In AL amyloidosis free light chains, mostly λ, are prone to amyloid formation which causes deposition in target organs mostly kidneys, heart, liver, gastrointestinal system, skin, fat and the nervous system (47). Direct myeloma cell infiltration of the kidney is also seen rarely.

MM patients most commonly present with bone pain and the most common sites of bone disease include the skull, vertebral column, ribs, pelvic, femoral and humeral bones (48). Lytic lesions, pathological fractures and osteoporosis are seen in simple xrays or computerized tomography (CT) and diffuse or focal patterns of BM infiltration can be seen in Magnetic resonance imaging (MRI) (49). Lytic lesions are the result of increased osteoclast and decreased osteoblast number and activity as the balance of bone metabolism is affected by the presence of myeloma cells in the bone marrow. (50). There is activation of the receptor NF-κB (nuclear factor- kappa B), RANKL and of osteoprotegerin (OPG). At the same time Wnt pathway inhibitors like Dickkpof-1 and sclerostin are activated and inhibit obsteoblastic activity.

Bone resorption leads to hypercalcemia. The incidence of hypercalcemia has decreased in more recent years secondary to more timely diagnoses of the disease. Hypercalcemia leads to renal impairment, polyuria, polydipsia, constipation and altered mental status.
Abnormalities in cellular and humoral immunity and immunoparesis caused by suppression the non-involved immunoglobulins makes MM patients susceptible to infection. They are particularly susceptible to infection by streptococcus pneumonia and Haemophilus influenza. (51). Coagulation abnormalities are also seen in MM either as venous thromboembolism events or hemorrhagic events. About 7% will present with hyperviscosity due to very high levels of immunoglobulins. (42).

1.3 Diagnosis, Laboratory work up and disease staging:

Diagnostic criteria

CRAB features

- Hypercalcemia: serum calcium levels of >1mg/dl higher than upper limit of normal levels (>11mg/dl)
- Renal insufficiency: Creatinine clearance of <40ml/min or serum creatinine levels of >2mg/dl
- Anemia: Hemoglobin levels of >2g/dl below the lower limit of normal (<10 g/dl)
- Bone lytic lesions: presence of one or more lytic lesions detected by conventional radiology, CT imaging (low dose CT) or PET-CT

Myeloma Defining events (MDE)

- CRAB features
- A clonal bone marrow plasma cell percentage (BMPC) of ≥60%
- An involved to uninvolved serum free light chain ratio of ≥ 100
- Two or more focal lesions on MRI

	MGUS	SMM	MM
Serum monoclonal protein levels	<3 g/dl And	≥3g/dl And/or	-
Clonal BMPC infiltration	<10%	10-60%	≥10% or biopsy proven plasmacytoma
Symptomatology	Absence of CRAB	Absence of MDE or amyloidosis	Presence of MDE

Table 2: International myeloma working group definition and diagnostic criteria for Multiple Myeloma

The International Myeloma Working Group (IMWG) criteria are used for the diagnosis of Multiple myeloma. (1) (Table 2). They are based on the presence of monoclonal protein levels and bone marrow infiltration in addition to "myeloma defining events" which include CRAB features but also two or more focal lesions on MRI, clonal bone marrow plasma cell percentage >=60% and an involved-to-uninvolved serum free light chain ratio of >=100.

Initial assessment includes medical and family history in addition to physical examination. Family history should focus on first degree relatives with hematological malignancies and medical history on comorbidities that could affect treatment decisions. The following laboratory and imaging examinations are required for diagnosis and staging: (52):

- Full blood count and blood smear

- A complete biochemistry screen should also be performed, which includes liver function tests and renal function tests (including glomerular filtration rate, electrolytes, calcium, creatinine, lactate dehydrogenase and albumin levels, β2-microglobulin).
- Renal biopsy is recommended by the IMWG in cases when renal failure is not associated the typical patterns of cast nephropathy.

- Serum and urine electrophoresis, 24 hour urine collection and serum and urine immunofixation, quantitative immunoglobulin assay (IgG, IgA, IgM, IgD, IgE), serum free light chain assay which is particularly important in cases of oligosecretory or non-secretory MM. A subset of patients have myeloma defining events with normal sFLC ratio so monoclonal protein secretion is not a required criterion for diagnosis. Overall 97% of patient have abnormal levels of immunoglobulins in the serum, 75% have detectable monoclonal protein in urine. The most commonly involved subtype is IgG in 60% , IgA in 20%, IgD in 2%. Light chain only involvement is seen in 15% of patients.
- Bone marrow aspirate and/or biopsy are required to determine that the percentage of clonal cells in the bone marrow is ≥ 10%. Clonality is established via immunohistochemistry using CD138 stains or via immunoperoxidase staining or immunofluorescence. Other methods of clone identification include immunophenotyping via flow cytometry.
- Bone marrow aspirate for cytogenetic testing is required for risk stratification of the disease and is necessary in all MM patients. (53) Del(17p), t(4;14), t(16;14) are required for prognostic staging of the disease. In terms of prognosis Del(17p), t(4;14) are considered to be the most informative markers. Karyotype analysis can also differentiate between hyperdiploid from non-hyperdiploid patients.

Radiological assessment:

- According to the new IMWG criteria bone disease evaluation can include the use of skeletal survey with plain X-rays, CT imaging or ^{18}F-fluorodeoxyglucose PET-CT. The aim is to use more sensitive techniques such as CT or PET-CT as lesions as small as >5mm indicate MM but the exact modality used is often determined by availability and resources. MRI of the thoracic or lumbar spine, or whole body MRI is required when MM is suspected in the absence of CRAB. Focal lesions in whole-body MRI in

patients with SMM was associated with shorter median time to progression compared to patients with no focal lesions. (54)

1.4 Prognostic factors and risk stratification:

The staging system most widely used is the International staging system (ISS). (55) The basis of the ISS is albumin and β2 microglobulin. The Revised-ISS includes cytogenetic abnormalities and LDH levels. (56) (Table 3) Currently the ISS is not used to determine management strategies. Between 10-18% of patients are classified as R-ISS III. According to the IMWG t(4;14), t(14;16), t(14;20) and del(17/17p), in addition to any non-hyperdiploid karyotype are considered to be high-risk cytogenetic factors in patients with multiple myeloma. (57) Given the better outcomes of patients with t(4;14) with proteasome inhibition some risk stratification systems associate it with intermediate risk disease. Combinations of two or more of any of the cytogenetic abnormalities render the patient ultra-high risk and are

	Stage	**Criteria**	**Median OS (months**
ISS stage	I	β2microglobulin <3.5 mg/l serum albumin ≥3.5 gr/dl	62
	II	Neither I or III	44
	III	β2 microglobulin ≥5.5 mg/l	29
R-ISS	I	ISS I Standard risk cytogenetics using FISH normal LDH levels	Not reached
	II	Neither I nor III	83
	III	ISS III High risk cytogenetics using FISH and including t(4;14), t(14;16) or del(17/17p), and/ OR high LDH	43
Other prognostic factors		• Circulating clonal plasma cells • Extramedullary disease • High plasma cell proliferative rate • High risk gene expression signatures • TP53 mutations • Renal failure • Poor PS • Immunoparesis • Plasma-blastic morphology	

Table 3: International Staging system (ISS) and revised-ISS as a prognostic system for multiple myeloma

associated with less than 2 year survival. (57) In the MRC Myeloma IX study these comprised 15% of patients. The Myeloma Genome Project built on the above scores and incorporated Next Generation Sequencing (NGS) and structural abnormalities to better define risk. The highest risk patients are referred to as "double hit" myeloma, meaning patients with two "hits" to the same gene, either loss of both alleles of *TP53* (by mutation, deletion or both) or with two extra copies of 1q resulting in amplification rather than single gain. About 6-10% of patients will be double hit and it seems that it has a greater prognostic power than R-ISS. (58)

1.8 Precursor states and risk of progression:

1.8.1 Monoclonal gammopathy of undetermined significance (MGUS):

IgM MGUS	All three criteria must be met: • Serum IgM monoclonal protein <3mg/dL • Bone marrow lymphoplasmacytic infiltration <10% • No evidence of anemia, constitutional symptoms, hyperviscosity, lymphadenopathy, or hepatosplenomegaly that can be attributed to the underlying lymphoproliferative disorder
Non IgM MGUS	All three criteria must be met: • Serum (non- IgM subtype) monoclonal protein <3mg/dL • Bone marrow lymphoplasmacytic infiltration <10% • Absence of end-organ damage such as hypercalcemia, renal insufficiency, anemia, and bone lesions (CRAB) that can be attributed to the plasma cell proliferative disorder
Light-chain MGUS	All criteria must be met: • Abnormal FLC ratio (<0.26 or >1.65) • Increased level of involved light chain (increased κ FLC in patients with FLC ratio >1.65 and increased λ FLC in patients with FLC ratio <0.26) • No immunoglobulin heavy-chain expression on immunofixation • Absence of end-organ damage that can be attributed to the plasma cell proliferative disorder • Clonal bone marrow plasma cells <10%* • Urinary monoclonal protein <500 mg per 24 h

Table 4: Monoclonal gammopathy of undetermined significance (MGUS) subtypes based on the IMWG criteria

MGUS is a premalignant clonal plasma cell disordered characterized by the presence of monoclonal (M)protein, <10% of clonal plasma cells in the bone marrow and absence of the features that define MM or related lymphoplasmacytic malignancies. Three types of MGUS are identified based on the type of the secreted monoclonal protein. (table 4) It is a requisite precursor of MM but also of immunoglobulin light-chain (AL) amyloidosis and Waldenström macroglobulinemia (WM). It is detected in 4% of individuals ≥50 years of age and ~5% of individuals ≥70 years of age and there is earlier age of onset and higher incidence in blacks than in whites. (59) There are three subtypes of MGUS with a distinct features and characteristics. (1)

The progression from the premalignant state of MGUS to MM is the consequence of a series of oncogenic events with genetic changes that promote myelomagenesis and changes in the complex interactions with the microenvironment. Several studies have demonstrated that the characteristic genetic abnormalities of MM are present also in patients with MGUS. Kyle et al reported that cells in IgG and IgA MGUS arise from mature, somatically mutated post-switch plasma cells and about 50% of cases will have evidence of translocation of the Ig heavy-chain region at 14q32. Abnormal serum free light chain ratio, isotype, monoclonal protein concentration, serum free light chain ratio (sFLC) and immunoparesis are considered risk factors for progression. (9) The Mayo Clinic model for MGUS risk progression and follow up combines the type of the Mprotein, its value and the serum FLC ratio. Non-IgG Mprotein, Mprotein > 1.5g/dl and abnormal FLC ratio are considered adverse prognostic features. (60) Another model has been proposed by the Spanish group PETHEMA. They used immunophenotyping with multiparameter flow cytometry to identify aberrant plasma cells (aPC) in the BM of 407 MGUS patients and demonstrated that a ratio of aPC/BMPC of >95% was an independent risk factor for progression in MGUS (and also SMM). DNA aneuploidy (hypo or hyperdiploidy) were also found to be independent prognostic factors for risk of progression. A prognostic index was formed by combining aneuploidy with aPC/BMPC >95%. (61) Full work-up of the patient with MGUS is similar to that of the patient with MM. Recommendations vary on the need for bone disease assessment. (62) (63)

Most groups do not recommend skeletal survey but there may be a place for bone mineral density testing using DEXA scan as MGUS patients with osteoporosis might benefit from administration of bisphosphonates. Follow up of patients with MGUS should include serum and urine electrophoresis, serum immunoglobulin and free light chain ratio usually every 4-6 months over the first 2 years and then every 1-2 years but recommendations vary. Bone marrow biopsy is not usually recommended at follow up unless the patient has high risk features.

1.8.2 Smoldering multiple myeloma (SMM):

SMM is an intermediate stage between MGUS and SMM. To diagnose a patient with SMM both of the following criteria must be met: a) serum monoclonal protein (IgG or IgA) ≥ 3 g/dL, or urinary monoclonal protein ≥ 500 mg per 24 h and/or clonal bone marrow plasma cells 10%-60% and b) absence of myeloma-defining events or amyloidosis. The annual risk of progression is 10% per year for the first 5 years, 5% per year during the subsequent 5 years and 1% per year after 10 years. (7) The Mayo Clinic model and the Spanish models described above for MGUS risk assessment are also used to classify SMM as low, intermediate and high risk and to determine risk of progression and follow up. These have been validated in prospective trials but newer risk models that incorporate novel clinical and biological features are emerging. (64)

The Mayo Clinic model categorizes patients into three risk categories using M-protein (≥3 g/dL), BMPC% (≥10%), and the ratio of involved to uninvolved serum free light chains (sFLC) (≥8). A 76% risk of progression in 5 years among those with all three of the above characteristics is estimated. (7) The Spanish model uses the proportion of BMPCs with aberrant PC phenotype on flow cytometry (≥95%) and reduction in uninvolved immunoglobulins (immunoparesis) to identify high-risk patients. (61) Abnormalities detected on imaging of spine or whole body using magnetic resonance imaging (MRI), and underlying cytogenetic abnormalities also guide clinicians in identifying high-risk patients. (54) (65)

Other risk factors that have been examined include IgA (vs IgG) isotype, proteinuria, circulating plasma cells and a high proliferative rate of bone marrow plasma cells. Two studies showed that that the presence of deletion 17p or t(4;14) is associated with the shortest time to progression (TTP) and that trisomies were a risk factor for progression from SMM to MM. Gains of 1q21 were also associated with increased risk for progression among patients with SMM. (66)

A recent risk stratification model which incorporates the revised IMWG diagnostic criteria was published in 2018 by Lakshman et al. (67) BMPC% > 20%, M- protein > 2g/dL and FLCr > 20 independently predicted shorter time to progression (TTP). Patients were stratified in three risk groups: low risk (none of the three risk factors) intermediate risk (one of the three risk factors) and high risk (≥2 of the three risk factors) and median TTP were 110, 68, and 29 months respectively.

Low risk SMM: Absence of the high-risk factors which include serum monoclonal protein levels of ≥3. 0 g/dl, non-IgG monoclonal protein, and serum involved/uninvolved free light-chain (FLC) ratio of ≥8 but <100. The probability of progression at 5 years is only 8% and these patients behave like MGUS. No risk factors of PETHEMA and 1 risk factor of Mayo clinic score.

Intermediate risk: Have some high-risk features and are probably the true SMM population. The risk of progression at 5 years is 42% and should be followed up every 3 months for the first year and then every 6 months. Two risk factors of Mayo clinic and 1 of PETHEMA.

High risk: These patients have three risk factors of Mayo clinic model and 2 of PETHEMA model. Rate of progression is >75% at 5 years and 50% at 2 years. Close follow is required. These patients are candidates for enrolment in clinical trials and SMM treatment. Can be considered as “early myeloma” in future definitions.

The current standard practice is to observe patients with SMM, a ”watch-and-wait” strategy. This is a paradigm which may soon change as many groups have attempted to answer the question whether early treatment of high-risk SMM might prolong the time to development of MM. The therapeutic approach to the SMM patient first to prevent progression and second to achieve complete remission, clone eradication and eventually

disease cure. To apply such an approach it is crucial to be able to identify the SMM patients who would benefit from such an intervention.

The first studies to examine the hypothesis whether early intervention would prevent progression go as far back as 1990s with the use of melphalan and prednisone and later with bisphosphonates or thalidomide. The phase III RCT by the PETHEMA group showed a significantly longer time to progression to MM in patients with high risk SMM treated with lenalidomide and dexamethasone compared to patients who were in the observation only arm after a median follow up of 40 months. At 75 months progression to MM was 86% in the observation arm compared to 39% in the Rd arm. (68) Several other trials are currently investigating the role of novel agents in this patient population (celexicib, lenalidomide alone, anti-KRI monoclonal antibody, BHQ880, elotuzumab, siltuximab, carfilzomib plus lenalidomide and dexamethasone, daratumumab).

1.9 Current goals of therapy and Response Criteria:

Achieving a deep a lasting remission is the current goal of treatment and as new drugs are being developed and incorporated into the treatment paradigm for the disease, deeper and more sustainable remissions are being achieved. The International Myeloma Working Group (IMWG) has published response criteria which were updated in 2011 to recognize stringent complete remission (sCR) (69) and then in 2016 to incorporate guidelines for the assessment of Minimal Residual disease (MRD).(70) sCR is a deeper remission than CR as it requires normalization of serum light chains and absence of clonal plasma cells using immonhistochemical stains on bone marrow biopsy versus CR which requires negative serum and urine immunofixation and less than 5% of plasma cell on BM biopsy irrespective of clonal restriction. While sCR is a deeper remission than CR most patients still have significant number of tumor cells present and additional tools are needed to measure lower levels of residual disease – Minimal residual disease (MRD). The two most widely used methods for MRD assessment are multi-color flow cytometry (MFC) and next generation sequencing (NGS). The two techniques have their advantages and limitations. Current MFC

techniques have been reported to have a limit of detection of 1 in 10^5 cells with reports of some techniques identifying as few as two tumour cells in 10^6 . (71) NGS can detect disease at a level of 1 cell in 10^6 cells. (72) A baseline sample is required for NGS to detect the sequence of interest but with MFC there is need for real-time analysis. Imaging techniques have also been used to detect residual disease and their use has been incorporated in the response criteria. MRD assessment and endpoints have been increasingly incorporated into clinical trials and MRD (-) is often used as the primary endpoint. Its use as a surrogate of OS can significantly shorten the time to gain approval for new therapies. (see table 5). (70)

Response criteria	
IMWG MRD criteria (requires a complete response as defined below)	
Sustained MRD-negative	MRD negativity in the marrow (NGF or NGS, or both) and by imaging as defined below, confirmed minimum of 1 year apart. Subsequent evaluations can be used to further specify the duration of negativity (eg, MRD-negative at 5 years
Flow MRD-negative	Absence of phenotypically aberrant clonal plasma cells by NGF‡ on bone marrow aspirates using the EuroFlow standard operation procedure for MRD detection in multiple myeloma (or validated equivalent method) with a minimum sensitivity of 1 in 10^5 nucleated cells or higher
Sequencing MRD-negative	Absence of clonal plasma cells by NGS on bone marrow aspirate in which presence of a clone is defined as less than two identical sequencing reads obtained after DNA sequencing of bone marrow aspirates using the LymphoSIGHT platform (or validated equivalent method) with a minimum sensitivity of 1 in 10^5 nucleated cells or higher
Imaging-positive MRD-negative	RD negativity as defined by NGF or NGS plus disappearance of every area of increased tracer uptake found at baseline or a preceding PET/CT or decrease to less mediastinal blood pool SUV or decrease to less than that of surrounding normal tissue
Standard IMWG response criteria	
Stringent complete response	Complete response as defined below plus normal FLC ratio and absence of clonal cells in bone marrow biopsy by immunohistochemistry (κ/λ ratio ≤4:1 or ≥1:2 for κ and λ patients, respectively, after counting ≥100 plasma cells
Complete response	Negative immunofixation on the serum and urine and disappearance of any soft tissue plasmacytomas and <5% plasma cells in bone marrow aspirates
Very good partial response	Serum and urine M-protein detectable by immunofixation but not on electrophoresis or ≥90% reduction in serum M-protein plus urine M-protein level <100 mg per 24hours
Partial response	≥50% reduction of serum M-protein plus reduction in 24 h urinary M-protein by ≥90% or to <200 mg per 24 h. If the serum and urine M-protein are unmeasurable, a ≥50% decrease in the difference between involved and uninvolved FLC levels is required in place of the M-protein criteria; if serum and urine M-protein are unmeasurable, and serum-free light assay is also unmeasurable, ≥50% reduction in plasma cells is required in place of M-protein, provided baseline bone marrow plasma-cell percentage was ≥30%. In addition to these criteria, if present at baseline, a ≥50% reduction in the size (SPD) of soft tissue plasmacytomas is also required
Minimal response	Minimal response ≥25% but ≤49% reduction of serum M-protein and reduction in 24-h urine M-protein by 50–89%. In addition to the above listed criteria, if present at

	baseline, a ≥50% reduction in the size (SPD) of soft tissue plasmacytomas is also required
Stable disease	Not recommended for use as an indicator of response; stability of disease is best described by providing the time-to-progression estimates. Not meeting criteria for complete response, very good partial response, partial response, minimal response, or progressive disease
Progressive disease	Any one or more of the following criteria: Increase of 25% from lowest confirmed response value in one or more of the following criteria: • Serum M-protein (absolute increase must be ≥0.5 g/dL); • Serum M-protein increase ≥1 g/dL, if the lowest M component was ≥5 g/dL; • Urine M-protein (absolute increase must be ≥200 mg/24 h); • In patients without measurable serum and urine M-protein levels, the difference between involved and uninvolved FLC levels (absolute increase must be >10 mg/dL); • In patients without measurable serum and urine M-protein levels and without measurable involved FLC levels, bone marrow plasma-cell percentage irrespective of baseline status (absolute increase must be ≥10%); • Appearance of a new lesion(s), ≥50% increase from nadir in SPD of >1 lesion, or ≥50% increase in the longest diameter of a previous lesion >1 cm in short axis; • ≥50% increase in circulating plasma cells (minimum of 200 cells per µL) if this is the only measure of disease • Development of hypercalcemia (corrected serum calcium > 11.5 mg/dL or 2.65 mmol/L) that can be attributed solely to the plasma cell proliferative disorder
Clinical relapse	Clinical relapse requires one or more of the following criteria: • Direct indicators of increasing disease and/or end organ dysfunction (CRAB features) related to the underlying clonal plasma-cell proliferative disorder. • Development of new soft tissue plasmacytomas or bone lesions (osteoporotic fractures do not constitute progression); Definite increase in the size of existing plasmacytomas or bone lesions. A definite increase is defined as a 50% (and ≥1 cm) increase as measured serially by the SPD of the measurable lesion; • Hypercalcaemia (>11 mg/dL) • Decrease in haemoglobin of ≥2 g/dL not related to therapy or other non-myeloma-related conditions • Rise in serum creatinine by 2 mg/dL or more from the start of the therapy and attributable to myeloma • Hyperviscosity related to serum paraprotein

Table 5: International Myeloma Working Group response criteria for Multiple Myeloma: MRD: minimal residual disease ;NGF: next generation flow ;NGS: next generation sequencing ; SPD: summary of product dimensions.

1.10 Current treatment:

1.10.1 Newly diagnosed Multiple myeloma patient

Currently used drugs in MM	
Proteasome inhibitors	Bortezomib
	Carfilzomib
	Ixazomib
Immunomodulatory drugs	Thalidomide
	Lenalidomide
	Pomalidomide
Monoclonal antibodies	Daratumumab (anti-cd38)
	Elotuzumab (anti-SLAMF7)
Histone deacetylase inhibitor	Panobinostat
Alkylating agents	Melphalan
	Cyclophosphamide
	Bendamustine
Others	Dexamethasone
	Prednisone
	Cisplatin
	Etoposide
	Doxorubicin

Table 6: Approved class agents and drugs for the treatment of multiple myeloma

The therapeutic armamentarium in multiple myeloma has expanded substantially over the last decades with several classes of agents available in the initial diagnosis and the relapsed disease setting. (table 6) The therapeutic approach is designed based on the patients age, comorbidities, preferences, performance status, disease risk stratification and frailty status. Traditionally, the initial decision on the suitability of the MM for autologous hemopoietic stem cell transplantation (ASCT) was a major determinant of the selected treatment strategy. The increasingly improved tolerability of anti-myeloma agents has lead over the years to a convergence of the treatment approaches in the two patient groups but transplant-eligibility still remains a substantial factor in the final decision of the treatment approach. Age has been the major determinant of ASCT eligibility and most randomized clinical trials have included patients ≤ 65 years old. Emerging data however increasingly support physiological rather than chronological age as the most significant factor. (73) (74) Comorbidities are the second major determinant and there is agreement that patients with substantial cardiac or pulmonary disorders should not be offered ASCT. Renal insufficiency event for patients on hemodialysis is not an exclusion criterion.

The combination of proteasome inhibitor(PI) and immunomodulatory agent (IMiD) is considered currently best clinical practice for the newly diagnosed multiple myeloma patient (NDMM). The combination of bortezomib, lenalidomide and dexamethasone (VRD) should be offered to all patients who can tolerate a multiagent combination based on the results of the phase III trial that compared VRD to lenalidomide and dexamethasone (Rd) in NDMM and showed an improvement in progression free survival (PFS). (75) In the presence of acute renal failure or when lenalidomide is absent, other bortezomib- containing regimens such as bortezomib- thalidomide-dexamethasone (VTd) or bortezomib-cyclophosphamide (VCd) can be used instead of VRd. Based on data from clinical trials, patients who received VTD versus VD had a PFS advantage but not an overall survival (OS) advantage.(76) Bortezomib plus alkylating agents like VCD or bortezomib with melphalan combinations are alternatives mostly for the transplant ineligible patient and clinical trial data have demonstrated low toxicity and high efficacy. (77) Triplet combinations are standard practice but doublet combinations are an option in patients who are frail. In the FIRST trial, transplant-ineligible patients who received Rd until disease progression versus MPT demonstrated improved PFS. (78) Frailty status assessment and optimal treatment choice in the older patients is crucial. (79) Newer options for initial therapy combinations include carfilzomib-lenalidomide-dexamethasone (KRd), daratumumab, lenalidomide, dexamethasone (DRd) and daratumumab plus VRd.

A multi-step treatment approach is currently followed for the NDMM patient. Following 3-4 cycles of induction treatment patients who are suitable candidates for ASCT will proceed to stem cell harvest. Peripheral stem cell collection is usually performed with growth factor support (granulocyte colony- stimulating growth factor) following a conditioning regimen chemotherapy and then administration of myeloablative conditions and reinfusion of the collected stem cells. The aim of ASCT in MM is to deepen the response to treatment and to improve response duration. ASCT has been incorporated in clinical practice for over 20 years as data from phase III clinical trials demonstrate an OS advantage even in the era of novel agent combinations. (80) (81)

Induction treatment is administered for 8-12 cycles in the transplant ineligible patient. Maintenance with lenalidomide has become commonplace after initial therapy for both transplant ineligible and eligible patients. In a meta-analysis of several phase III randomized trials a significant improvement in PFS and OS is seen with lenalidomide maintenance compared with placebo or no therapy.(82) (83) The benefit is however limited for high risk patients and bortezomib-based maintenance should be considered. The role of consolidation treatment following ASCT and prior to maintenance is also being investigated in many ongoing clinical trials. A recent trial by the Intergroupe Francophone du Myelome compared early versus delayed ASCT in patients treated with VRd followed by lenalidomide maintenance and patients were randomized to receive either VRd (3 cycles) followed by ASCT and then VRd consolidation (2 cycles) versus VRD x 8 cycles with ASCT reserved for relapse and both arms received lenalidomide for 1 year. (84) A significant improvement of PFS was seen with early ASCT but results in OS are awaited. Tandem ASCT has been compared to single ASCT and most studies demonstrate a PFS advantage but not an OS advantage except for patients with high-risk genetic factors such as del(17p) and t(4;14). (85, 86) Allogeneic transplantation is still investigational but can be considered for young patients with high-risk disease in early relapse.

The following is a recommended approach algorithm depending on the risk characteristics of the disease at first line (Kumar et al 2017 (21).

Transplant ineligible:

- Standard risk disease (t(11;14) t(6;14) and trisomies): Vrd or Rd for 12 months and then lenalidomide maintenance until disease progression for a minimum of 1 year.
- Intermediate risk (t(4;14): VRd for 12 months and then bortezomib based maintenance until disease progression or as tolerated.
- High risk (del(17p), t(14;16) and t(14;20): VRd for 12 months and then bortezomib and lenalidomide or bortezomib only maintenance until disease progression as tolerated.

Transplant eligible patients:

- Standard risk disease (t(11;14) t(6;14) and trisomies): 4 cycles of Vrd, then collect stem cells, then ACST followed by R maintenance for 2 years
-
- Intermediate risk (t(4;14): 4 cycles VRd, then stem cell collection and ASCT or tandem ASCT followed by bortezomib maintenance for 2 years

- High risk (del(17p), t(14;16) and t(14;20): 4 cycles VRd or KRd or enroll in clinical trial, then stem cell collection and ASCT or tandem ASCT followed by VR or KR until disease progression

1.10.2 Relapsed MM:

Despite the effectiveness of frontline treatment, relapse is inevitable. With every relapse response duration is usually shorter reflecting the development of drug refractoriness and reduced drug efficacy by the increasing genomic complexity of the monoclonal plasma cells via the acquisition of mutations and epigenetic alterations. New agents are continuously being developed and being explored in clinical trials in an attempt to overcome disease resistance in the MM patient. (36) The choice of treatment regimen at relapse is complicated and affected by many factors including timing of relapse, response to prior therapy, aggressiveness of relapse and performance status (TRAP) (table 7). (87)

Current therapy	Fit patients Consider salvage ASCT if no ASCT at first line or if they had >18 months unmaintained response or >36 months maintained response in first ASCT	Frail patient Can consider triplet combinations for frail patients also with adequate dose reductions
Patients who are on maintenance therapy		
Thalidomide	Bortezomib, carfilzomib, Ixazomib, Elotuzumab, or daratumumab combined with lenalidomide	Lenalidomide, Bortezomib, carfilzomib, Ixazomib or daratumumab combined with dexamethasone
Lenalidomide	Bortezomib, carfilzomib, Ixazomib, Elotuzumab, or daratumumab combined with pomalidomide or daratumumab with bortezomib	Bortezomib, carfilzomib, Ixazomib or daratumumab combined with dexamethasone
Bortezomib	Carfilzomib, Elotuzumab, daratumumab combined with lenalidomide	Lenalidomide, carfilzomib or daratumumab combined with dexamethasone
Patients who are not on maintenance therapy		
NA	Bortezomib, carfilzomib, Ixazomib, Elotuzumab, or daratumumab combined with lenalidomide and dexamethasone or daratumumab with bortezomib	Doublets of lenalidomide, bortezomib, Ixazomib, carfilzomib or daratumumab with dexamethasone

Table 7: Algorithm for the choice of treatment combination at the time of relapse

At the time of the relapse it should be decided whether treatment needs to be initiated. Biochemical only progression with slow increase in the monoclonal protein levels does not require immediate treatment initiation and close monitoring may be sufficient. The presence of CRAB features, rapid monoclonal protein increase or neurological symptoms are however indications for immediate treatment initiation. There is no simple algorithm for agent selection and the optimal combination and best sequence of treatments remains unknown. The choice of treatment combination should take into account the risk status and symptoms of the patient, the presence of high risk genetics and disease risk stratification, prior treatment regimens, prior sensitivity to drugs and response duration, prior toxicities and adverse effects together with performance status, frailty and patient wishes. Treatment goals can vary significantly among patients. In young fit patients aggressive combinations that can achieve MRD negativity and enrollment in clinical trials is usually appropriate. In frail patients the goal is usually to achieve disease stabilization and minimum toxicity .

Patients eligible for transplantation should be considered for the procedure if they have never had one before or if they had a very good remission duration with the first transplantation. VRd, VCd and VTd are active regimens also in relapsed disease. Daratumumab based combinations have shown efficacy: DRd, DVd and daratumumab, pomalidomide, dexamethasone (DPd). Other options include KRd, Ixazomib lenalidomide, dexamethasone (IRd), Elotuzumab, lenalidomide and dexamethasone (ERd) and various pomalidomide-based regimens such as DPd and carfilzomib, pomalidomide and dexamethasone (KPd). Anthracycline- containing regimens may be useful for aggressive relapses. Other drugs to consider are panobinostat, a pandeacetylase inhibitor and bendamustine containing regimens such as bendamustine, lenalidomide and dexamethasone or bendamustine, bortezomib and dexamethasone. Venetoclax appears to have single-agent activity in patients with t(11;14) subtype of MM.

At first relapse triplet combinations are usually opted for. The most impressive results are from the Pollux trial in which 62% of patients treated with DRd for 1st relapse remained in remission at a median of 36 month follow up. (88) In the Castor trial 68% of patients who

received DVd remained in remission during 18 month follow up. (89) The OptimisMM which compared pomalidomide plus Vd vs Vd and the Aspire trial which compared KRd to Rd also reported improved PFS and OS with the use of triplet versus doublet combinations. (90) Treatment is continued until disease progression. At second relapse usually the drug class is switched or second or third generation agents are utilized. Choosing the appropriate agent combinations becomes increasingly difficult and often next generation IMiDs (pomalidomide) and PIs (carfilzomib) are selected. Patients are also enrolled in clinical trials with novel agents.

1.10.3 Supportive management:

Bisphosphonates delay the progression of lytic bone lesions and prevent fractures and are indicated for all MM patients with bone involvement. Dose adjustments are required for mild renal impairment but bisphosphonates are contraindicated in patients with significant renal dysfunction. Bisphosphonates are also indicated for patients with hypercalcemia. Zoledronic acid is usually the agent of choice with the most challenging complication being jaw osteonecrosis. The current recommendation by the IMWG and the American Society of Clinical oncology is to avoid using bisphosphonates for more than two years at initial diagnosis but to restart with relapse. Newer agents such as denosumab are under investigation. Orthopedic surgery complemented with radiotherapy may be indicated in patients with long bone pathological fractures or with vertebral compression fractures. Local radiotherapy is also indicated for spinal cord compression. Anemia can be managed with erythropoetic stimulating factors but transfusions may also be required. Influenza and pneumococcal vaccinations are recommended and acyclovir or valacyclovir for herpes zoster virus prophylaxis is recommended for patients on PI-based regimens. Prophylaxis of infection with the administration of antibiotics remains controversial. It might be beneficial during the first months of treatment initiation particularly when patients receive lenalidomide or pomalidomide. Risk assessment for venous thromboembolism is also required and use of aspirin for low risk patients and LMWH or full dose warfarin for higher risk patients is currently recommended when patients receive IMiDs. (91, 92)

1.10.4 Future approaches:

Myeloma remains an incurable disease despite significant progress in the disease understanding and advances in therapy. The clinical course of the disease remains very heterogenous due to underlying molecular variation and currently available tools for risk assessment can only identify a proportion of patients who will have an adverse outcome.

It is increasingly argued that in the future treatment should become personalized and tailored to risk assessment. Current trials are evaluating such an approach. Early steps towards this approach include gene expression signatures and mutation panels that can identify new mutations. The tumor microenvironment also plays a significant role in the disease phenotype. Understanding of the complex interplay of its component parts will add significant knowledge to disease characteristics and contribute to the development of effective treatment mechanisms. Individual molecular features or “biomarkers” need to be identified that are of “prognostic” and “predictive” value.

The success of immune-based therapies has directed research efforts also to the immune profile of patients with MM. Monoclonal antibodies, check-point inhibitors and T-cell therapies are immune therapies which have shown significant promise. (table 8) Daratumumab is an antibody which targets the CD38 antigen on the myeloma cells and in combination with IMiDs or PIs has led to MRD negativity even in the relapsed setting. (88, 93)

Venetoclax is an inhibitor of the anti-apoptotic protein BCL-2 and data show that cells lines with t(11;14) show greater sensitivity to venetoclax which has been confirmed in clinical studies. Following the encouraging results of a phase I/II trial with venetoclax-VD in patients with RRMM the results of the phase 3 registration trial compared venetoclax, Vd versus Vd in early relapsed patients are awaited. Selinexor is a first-in-class, orally bioavailable, selective inhibitor of the nuclear transporter protein exportin 1 (XPO1). The phase II STORM study assessed selinexor in penta-refractory (bortezomib, carfilzomib,

lenalidomide pomalidomide, daratumumab) patients and an overall response rate (ORR) of 26.2% was reported.(94) A phase III registration trial (BOSTON) is comparing selinexor plus Vd versus Vd alone in early relapsed MM.

Small molecules	
Filanesib	Kinesin spindle protein inhibitor
Marizomib	Proteasome inhibitor
Selinexor	Inhibition of nuclear export protein
Ricolinostat	Histone deacetylase 6 inhibitor
Mefluflen	Alkylating agent
Venetoclax	Inhibition of bcl2
Immune therapies	
MOR202	antiCD38 monoclonal antibody
CC-92480	cereblon E3 ubiquitin ligase modulating drug (CELMoD)
Iberdomide	cereblon E3 ubiquitin ligase modulating drug (CELMoD
Durvalumab	PDL1 checkpoint inhibitor
Nivolumab	Anti-PD1 checkpoint inhibitor
CD19 CART cells	CD19
BCMA CART cells	BCMA
Pembrolizumab	Anti-PD1 checkpoint inhibitor
Isatuximab	antiCD38 monoclonal antibody

Table 8: Agents currently in clinical trials for multiple myeloma: BCMA: B cell maturation antigen, CAR: chimeric antigen receptor, PD1: programmed cell death protein 1, PDL1: programmed cell death ligand 1

Anti-BCMA monoclonal antibodies (B cell maturation antigen) have shown great promise in initial studies and include antibody drug conjugates (ADCs), bispecific T-cell engaging antibodies and chimeric antigen receptor T-cells (CAR-T cells). The antigen is expressed in the majority of malignant plasma cells with little off-target expression. GSK2857916 is a humanized anti-BCMA antibody that is conjugated to monomethyl auristatin-F, a

microtubule disrupting agent and induces cell death via antibody dependent cellular toxicity (ADCC), auristatin-mediated toxicity, BCMA receptor signaling inhibition and immunogenic cytotoxicity. The ORR to monotherapy from the phase I trial was impressive at 60% with median PFS of 7.9 months. Breakthrough status has been granted by the FDA to GSK285791 and a phase II study is underway. (95) Great caution is required with the adverse effects of these agents as they can be difficult or impossible to manage given the unknown interactions of these drugs with the immune system. There are more than 10 trials investigating CAR T-cells in MM. Results from bb2121, a BCMA targeted Phase I CAR-T cell study were recently presented and received with great enthusiasm as they reported an overall response rate (ORR) >95%) in patients receiving >150 x106 cells. The median PFS in the expansion cohort was 11.8 months. In another trial a dual BCMA epitope-binding CART cell, LCAR B38 M, led to an ORR of 80% with 68% of patients achieving CR. Caution is required with cytokine release syndrome, potential neurotoxicity. (96) Intervention at an earlier stage as discussed previously in the section on smoldering MM is increasingly favored by some myeloma clinicians.

2. Coagulation

2.1 Coagulation pathways

The blood vessel wall, circulating platelets, coagulation factors and coagulation inhibitors closely interact to achieve a fine balance between procoagulant and anticoagulant mechanisms and the normal hemostatic response to vascular damage.

There four phases of the hemostatic process include:

2.1.1 Endothelial injury and formation of the platelet plug

2.1.2 Propagation of the clotting process by the coagulation cascade

2.1.3 Termination of clotting by antithrombotic control mechanisms

2.1.4 Removal of the clot by fibrinolysis

2.1.1 Endothelial injury and formation of the platelet plug

The initial "platelet plug" formation depends on the complex interactions between platelets and vessel wall adhesive proteins. The vascular wall is lined by endothelial cells with antithrombotic properties which depends on the synthesis and secretion of platelet inhibitors, coagulation inhibitors, activators of coagulation, the function of nitric oxide, prostacyclin and the ectonucleotidase CD39, the presence of neutral phospholipids and negatively charged heparins. The subendothelial layer on the other hand is highly thrombogenic. It contains Von Willebrand factor (vWF), collagen and other proteins involved in platelet adhesion. The subendothelial connective tissue is separated from the circulating blood by the basement membrane of the vessel wall.

Collagen and tissue factor (TF) found in the endothelial cells becomes exposed to the circulating blood components once the vessel wall is breached and hemostatic mechanisms are activated. The initial trigger for platelet activation is collagen exposure whereas exposed TF initiates thrombin generation which will convert fibrinogen to fibrin and also activates platelets. Blood flow slowing to the site of the vessel wall breach is achieved by

vasoconstriction of the injured vessel and reflex constriction of the adjacent vessels allowing contact activation of platelets and coagulation factors.

Platelets and their role in thrombus formation:

Platelets are the second most abundant cell type in the blood, produced from megakaryocytes in the bone marrow. (97) Megakaryopoiesis, is primarily regulated by the cytokine thrombopoetin (TPO) which is mostly produce in the liver and also in the kidneys and skeletal muscles. TPO also drives thrombopoiesis, the production of platelets from mature megakaryocytes. Platelets are anuclear cells, contain functional organelles (endoplasmic reticulum, Golgi apparatus, mitochondria and granules) and play crucial roles in in hemostasis, repair and inflammation (98). Following vascular injury platelets adhere to collagen and vWF in the subendothelial tissue. (99) Platelets form the initial hemostatic plug that provides the surface for activated coagulation factors to assemble and leads to the formation of fibrin-stabilized platelet aggregates and clot retraction. Platelet plug formation includes platelet activation, platelet adhesion, platelet secretion and finally platelet aggregation.

a. Platelet activation: Thrombin and collagen are the most potent platelet activators while adenosine diphosphate (ADP) and epinephrine are relatively weak ones. The two mechanisms of pathway activation include:
    - The first pathway is independent of thrombin and involves the interaction of glycoprotein VI found on platelets with the collagen of the exposed vessel wall and of platelet glycoprotein Ib-X-IX with collagen-bound vWF which allow the adhesion of platelets to the site of injury. The platelet integrin $\alpha_2\beta_1$ plays a supportive role in this interaction.
    - The second mechanism depends on TF and does not require disruption of the endothelium. TF forms a complex with factor VIIa and the complex activates factor IX initiating a proteolytic cascade that generates thrombin. Platelets have a dual receptor system for thrombin with two distinct G-protein coupled protease activated

receptors (PAR-1 and PAR-4). PAR-1 is a high affinity receptor that mediates activation of platelets at low thrombin concentrations and PAR-4 a low affinity receptor that requires high levels of thrombin for activation. Thrombin activates platelets to release ADP, serotonin and thromboxane A2 which then activate other platelets amplifying the for thrombus formation.

Spatially, thrombin initially activates platelets in the core of the hemostatic plug and ADP then activates more loosely packed platelets in a shell overlying the core and thromboxane provides critical activation in the shell region. (100) (101)

b. Platelet adhesion: Following activation platelets undergo shape changes producing elongated pseudopods that make them extremely adhesive and increase surface area. Adhesion is primarily mediated by the binding of platelet surface receptor GPib/IX/V complex to VWF. (102)
c. Platelet secretion: Post adhesion degranulation with release of various factors takes place. Calcium and phospholipids that appear following platelet activation provide the surface for assembly of coagulation factors. Platelets release alpha (α) granules that contain VWF, P selectin, fibrinogen, fibronectin, factor V, factor VII, platelet factor IV, platelet derived growth factor and tumor growth factor α (103) and the delta or dense granules containing adenosine triphosphate (ATP), adenosine diphosphate (ADP) calcium, serotonin, histamine and epinephrine (103). Fibronectin and thrombospondin stabilize the platelet aggregate, ADP and serotonin activate and recruit additional platelets. Extracellular vesicles (EVs) are also released from platelets. These include exosomes and microvesicles (MVs) called microparticles (MPs) which contain mRNAs and miRNAs that can be delivered to target cells.
d. Platelet aggregation: VWF and fibrinogen bind to GPIIb/IIIa receptor once platelets are activated and undergo conformational changes and this leads to platelet spreading and clot retraction. Further platelets aggregate via TXA2 and ADP release and the initial platelet plug forms and seals of the breach of the vascular wall temporarily. GPIIb/IIIa receptors undergo a conformational change that allows

fibrinogen deposition and thrombin catalyzes the conversion of fibrinogen to fibrin adding to the stability of the platelet plug – secondary hemostasis. (103)

TXA2 and prostacyclin balance prevent clot extension and the patency of the vessel lumen is maintained.

2.1.2 Propagation of the clotting process by the coagulation cascade

Blood coagulation is a biological amplification system in which few initiation substances activate a cascade of reactions of circulating coagulation factor enzymes via proteolysis.. Thrombin generation is the end effect which converts soluble plasma fibrinogen into fibrin. Fibrin embeds in the platelet aggregates and converts the unstable primary platelet plug to stable hemostatic plug. Multicomponent macromolecular complexes form which facilitate the function of the active enzymes.

Thrombin:

Thrombin generation occurs in two waves of different magnitude. During the initiation phase small amounts are produced mediated by factor X activation by TF/ FVIIa which then leads to activation of factors VIII, V and XI in a feedback loop and a second larger thrombin burst with the amplification phase of the coagulation cascade which involves the assembly of multi-component complexes. The time course of thrombin generation is demonstrated by an initiation phase with the generation of a small amount of thrombin generation followed by the propagation phase when the bulk of thrombin is generated and finally cessation. (104) Standard laboratory tests detect the initial fibrin clot formation and measure primarily the initiation and not the propagation phase of clotting.

Coagulation enzymes: (table 9)

Coagulation enzymes are synthesized in the liver except from VWF which is synthesized in megakaryocytes, endothelium and subendothelial connective tissue, and factor VIII which is produced in liver endothelial cells. Clotting factors are classified into three categories; i)

fibrinogen family clotting factors ii) the vitamin K dependent clotting family (prothrombin, factor VII, IX and X) and (iii) contact family clotting factors. All the enzymes except factor XIII are serine proteases (the ability to hydrolyze peptide bonds depends on the amino acid serine

Clotting factor number	Clotting factor name	Function
I	Fibrinogen	Clot formation
II	Prothrombin	Activation of I, V,VII,VIII,XI, XIII, protein C, platelets
III	Tissue factor	Cofactor of VIIa
IV	calcium	Facilitates coagulation factor binding to phospholipid
V	Proacclerin labile factor	Co factor of X- prothrombinase complex
VI		
VII	Stable factor, procovertin	Activates factors IX, X
VIII	Antihaemophilic factor A	Cofactor of IX-tenase complex
IX	Antihaemophilic factor B or Christmas factor	Activates X, forms tenase complex with VIII
X	Stuart-Prower factor	Prothrombinase complex with factor V, activates factor II
XI	Plasma thromboplastin antecedent	Activates factor IX
XII	Hageman factor	Activates VII, XI, prokallikrein
XIII	Fibrin-stabilizing factor	Crosslinks fibrin
XIV	Prekallikrein (F Fletcher)	Serine protease zymogen
XV	HMWK-(F Fitzgerald)	Cofactor
XVI	vWF	Binds to VIII, mediates platelet adhesion
XVII	Antithrombin III	Inhibits IIa, Xa
XVIII	Heparin cofactor II	Inhibits IIa
XIX	Protein C	Inactivates Va, VIIIa
XX	Protein S	Cofactor for activated protein C

Table 9: Clotting factors (number, name and function)

at their active center).The majority are zymogens (precursors of proteolytic enzymes) and circulate inactive or as co-factors. Activation leads to post-translational modification which

enables binding of calcium and participation in the clotting cascade. The first 4 of the 12 originally identified factors are referred to by their common names: fibrinogen, prothrombin, tissue factor and calcium and are not assigned a numeral. The more recently discovered clotting factors (prekallikrein and high-molecular weight kininogen) have not been assigned Roman numerals.

Tissue factor:

Tissue factor or factor III is a membrane protein which initiates blood coagulation but also mediates intracellular signaling events important for angiogenesis, tumor progression and metastasis. (105) It is constitutively expressed on fibroblasts and pericytes in the adventitia and media smooth muscle cells of the vessel well but it is also expressed on many nonvascular cells. (106) The endothelium separates blood components from TF but a proportion is bloodborne and participates in physiologic and pathologic processes. (107) Tissue factor is also associated with circulating blood MPs (TF-MPs). During thrombus formation TF-MPs derived from monocytes bind to the thrombus via P-selectin glycoprotein ligand 1 (PSGL-1). TF on MPs does not initiate blood coagulation as it exists in a latent (encrypted form) that lacks coagulant activity. (108) (109)

Disulfide isomerase released by activated protein and endothelial cells is a protein required for TF activation, fibrin generation and platelet thrombus formation. (107, 110)
TF is the sole initiator of thrombin generation and fibrin formation (111) but the contact pathways of blood coagulation, a tool for in vitro studies of the coagulation cascade is not required for the initiation of hemostasis in vivo. One hypothesis regarding in vivo coagulation is that the activation of encrypted TF by protein disulfide isomerase is required to initiate coagulation. In the case of direct tissue damage TF may already exist in its active form and the isomerase may not be required.

Before thrombin generation, the TF pathway proceeding through factor IX or factor X is inefficient because factors VIII and V and circulating pro-cofactors required in the tenase and prothrombinase multi-component enzyme complexes are not yet available in their most active cofactor form. A small amount of thrombin is only formed. It converts factors VIII and

V to their cofactors forms and the tenase and prothrombinase complexes proceed to generate a large burst of thrombin. TF pathway is downregulated or inhibited by the action of the TF pathway inhibitor (TFPI), but thrombin generation proceeds without replenishing active tissue factor. (112, 113) The question of how thrombin is continuously generated in the absence of continued production of the active TF- FVIIa complex remains unresolved. In vitro studies have provided an explanation by the pathways described below of thrombin feeding to activate factors VIII and V.

The generation of thrombin in vivo is a complex network of amplification and negative feedback loops to ensure a localized and limited production. The generation of thrombin is dependent on three enzyme complexes each of which consists of a protease cofactor, phospholipids (PL) and calcium. Traditionally it has been distinguished into the intrinsic and extrinsic pathways both of which converge on factor X activation. This theory allows the understanding of in vitro coagulation tests but fails to incorporate the central role of cell-based surfaces in the in vivo coagulation process. (114)

Multicomponent complexes:

A total of four multicomponent macromolecular complexes play a major role in coagulation pathways: three procoagulant pathways (intrinsic and extrinsic X-ase and prothrombinase) and one anticoagulant complex all of which require the presence of PL and Ca2+.

- Extrinsic X-ase (ten-ase): activator factor VIIa as a protease, TF as the cofactor and factor X as the substrate. It activates both factor IX and X. (115)
- Intrinsic X-ase (ten-ase): factor IXa as the protease, activated factor VIII as the cofactor and factor X as the substrate. Factor IXa can be generated by the extrinsic or via activation of the intrinsic pathway either directly or indirectly via thrombin-induced activation of factor IX.
- Prothrombinase: factor Xa as a protease, factor Va as the cofactor and prothrombin as the substrate
- Protein C anticoagulant complex: thrombin as the enzyme (IIa) thrombomodulin as the cofactor and protein C as the substrate.

Coagulation cascade in vitro:

1. Initiation

Exposure of TF to factor VII/VIIIa leads to TF-VIIIa complex formation and the coagulation cascade is initiated. (108) (109) One to two percent of the total factor VII circulates in the activated form but does not express proteolytic activity unless bound to TF. The factor VIIa-TF (extrinsic factor Xase) complex activates both factor IX and factor X. Factor Xa in the absence of its cofactor forms small amounts of thrombin and prothrombin. This is insufficient to initiate significant fibrin polymerization but it activates factor V and factor VIII, platelets and cofactor XI.

2. Amplification

TFPI inactivates the initiation pathway or extrinsic Xase. In the amplification phase the intrinsic Xase formed by IXa and VIIIa (on phospholipid surface in the presence of Ca2+) activates sufficient Xa which forms the prothrombinase complex with Va, PL and Ca2+ resulting in the explosive generation of thrombin.

3. Propagation

The accumulated enzyme complexes (tenase and prothrombinase) on the platelet surface ensure continuous generation of thrombin and fibrin to form a sufficiently large clot.

4. Stabilization:

Thrombin generation leads to activation of factor XIII which covalently links fibrin polymers and provides stability to fibrin incorporated in the platelet plug.

2.1.3 Termination of clotting by antithrombotic control mechanisms

To limit the effect of thrombin to the site of injury naturally occurring anticoagulants in the body and the mechanisms of blood flow and fibrinolysis are important to control the extent of coagulation. TFPI is a protease inhibitor, a polypeptide synthesized mostly by the microvascular endothelium. Its concentration in plasma is very low (20% of total) and 80% is associated with low-density lipoproteins. It inhibits factor X activation by direct inhibition and also complexes with factor Xa to form the TFPI-FXa complex which inhibits TF/FVIIa

regulating the triggering mechanisms of the extrinsic pathway. It also inhibits VIIa and TF and limits the vivo pathway by forming a quaternary complex. (116, 117) Other thrombin inhibitors are heparin cofactor II, a1 macroglobulin and a1 antitrypsin. Another protein, heparin cofactor II also inhibits thrombin. A2 macroglobulins, α2antiplasmin, C1 esterase inhibitor and α1 antityrpsin also exert inhibitory effects on circulating serine proteases.

Antithrombin (AT) is a SERPIN (serine protease inhibitor) which binds and inactivates thrombin, factor IXa, Xa, Xia, XIIa by combining with them by peptide bonding to form high molecular weight stable complexes. The role of AT is enhanced by endogenous heparin and heparin sulfate but plasma concentration of heparin is low and does not contribute significantly to the in vivo activation of AT. AT binds coagulation factors in a ratio 1:1 and this complex is then removed by reticuloendothelial cells.

As clot formation progresses thrombin binds to thrombomodulin (TM). Binding leads to a change in the thrombin substrate specific, loss of its procoagulant function and ability to activate protein C. It therefore acts like a molecular switch. Protein C is vitamin K dependent serine protease with anticoagulant, profibrinolytic and anti-inflammatory properties. TM activated protein C (ACP) inhibits activated factors V and VIII. Its action is enhanced by another vitamin K dependent protein S, which binds protein C to the platelet surface. Protein S exists in plasma in both free and bound forms. The bound form is an inhibitor of the complement system and is upregulated in inflammatory states. Protein S also directly inhibits the prothrombinase complex directly. Endothelial protein C receptor (EPCR) also promotes protein C activation by localizing it to the endothelial surface and the thrombin-TM complex. Activated protein C is subject to inactivation by SERPINS like Antithrombin. (118)

Heparanase is a β-D-endoglucuronidase abundant in platelets that was discovered 30 years ago. It is an enzyme that cleaves heparan sulfate (HS) side chains on the cell surface in the extracellular matrix. It cleaves HS at a limited number of sites.

2.1.4 Removal of the clot by fibrinolysis

Plasminogen is a β—globulin proenzyme in blood and tissue fluid which is converted to the serine protease plasmin by activators either from the vessel wall (intrinsic) or from the tissues (extrinsic activation). Plasminogen binds fibrin and tissue plasminogen activator (tPA) which leads to conversion of the proenzyme plasminogen to active, proteolytic plasmin. Plasmin generation at the site of injury limits the extent of the evolving thrombus. tPA release is stimulated by thrombi, serotonin, bradykinin, cytokines and epinephrine following stimuli such as trauma, stress or exercise. Plasminogen is activated by urokinase which is present in high concentrations in the urine. tPA is responsible largely for the initiation of intravascular fibrinolysis and urokinase is the major activator of fibrinolysis in the extravascular compartment. The split products of fibrinolysis are also competitive inhibitors of thrombin and fibrin polymerization. A2antiplasmin inhibits any local free plasmin and tPA is inactivated by plasminogen activator inhibitor. Circulating plasmin is inactivated by potent inhibitors a2-antisplamin and a2-macroglobulin.

2.2 Microparticles and coagulation:

Microparticles (MPs) were first described by Chargaff and West in the 20th century as a "precipitable factor" present in plasma that promotes coagulation (119) and Wolf in 1962 described "platelet dust" that was formed as a result of platelet shedding which also exhibited procoagulant activity. (120) Microparticles are defined as heterogenous, submicron vesicles released from cell membranes in response to specific stimuli or apoptosis. (121) Many cells (platelets, RBCs, endothelial cells and leukocytes) shed their membrane fragments into the bloodstream in the form of microparticles during cell activation, injury or apoptosis. Platelet-derived MPs are the most abundant type and constitute 70-90% of all circulating MPs. Tumor cells are also capable of producing MPs that

appear in blood. (122) The formation and release of MPs occurs upon stimulation or induction of apoptosis and it is considered a primitive response to stress of eukaryotic cells which reflects a dynamic balance between cell proliferation, stimulation and death. (123) Upon stimulation, there is loss of the phospholipid asymmetry and cytoskeletal disruption which leads to membrane blebbing, MP formation and release. They are characterized and detected based on the antigens characteristic of their respective parental cells. They are produced by bidding and fission of the plasma membrane and they exhibit surface anionic phospholipids, cellular origin antigens, cytokines and matrix metalloproteinases and contain mRNAs and microRNAs. They are cleared from the circulation by phagocytosis but rapid clearance from the circulation may be also attributed to splenic removal and internalization by endothelial cells. MPs have physiological roles in multiple processes such as hemostasis, intercellular communication by transfer of cytoplasmic proteins and RNAs to recipient cells, modulation of innate and adaptive immune responses, angiogenesis, vascular and tissue repair, cancer metastasis via MMP activation, multidrug resistance and cell differentiation. (124) (125)

Newer data provide evidence for a direct link between VTE and microparticles as different phenotypes of MPs increase in states of thrombosis. (126) Their role in thrombogenesis can be summarized as: exposure of PS and TF vesiculation and MP-induced intercellular communication by cross-talk between inflammation and coagulation. (126)

The procoagulant activity of platelet MP surface is 50-100 fold higher than that of activated platelet surface. Their prothrombotic potential depends on: (1) their surface binding sites for coagulation factors (ii) the exposure of phosphatidylserine due to its translocation from the inner to the outer layer during platelet activation and the (iii) increased expression of surface tissue factor. (127, 128) The exposure of PS on the outer membrane acts as a catalytic surface for the assembly of enzymatic coagulation complexes that initiate and maintain coagulation.

Platelet- MPs are crucial in TF transfer from platelets to monocytes though P-selectin on PMPs and P-selectin glycoprotein ligand1 (PSGL1) on monocyte derives MPs. (107) Other

populations of MPs have been shown to display TF on their surface which has a high affinity for FVII/FVIIa and therefore TF-MPs bind these clotting factors and initiate the extrinsic pathway of the coagulation cascade. (129, 130) It is well known that activated monocytes and tumor cells and the primary sources of TF-bearing MPs in the blood stream. (131) (132) Newer data however suggest that MPs can initiate thrombin generation independently of TF and the extrinsic pathway through the intrinsic pathway and a FXII-dependent manner. (133)

There is also evidence regarding the ability of MPs to regulation coagulation through anticoagulant or fibrinolytic mechanisms. They have been shown to harbor functionally active TFPI on their membrane and support activated protein C and S, also expose fibrinolytic properties and support plasmin generation. (134) More recent studies also support an important role for MPs as significant mediators of intercellular communication. They contain antigens of their origin cell and can transfer these surface molecules to other cell types and organs and binding of MP to their specific counter receptor may activate intracellular signaling pathways. Cross talk between inflammation and hemostasis pathways is facilitated suggesting that events surrounding the release of endothelium derived MPs and its subsequent binding to monocytes might be involved in thrombogenesis. (135).

To date increased circulating MPs have been reported in many prothrombotic conditions including cancer and have been linked in some cases to the presence of a thrombotic event. These studies are retrospective or cross-sectional and need to be interpreted with caution. While there is an association between elevated MPs levels and prothrombotic conditions, data on a causal relationship between increased circulating MPs and thrombotic events in malignancy and other states are inconclusive so far.

3.0 Venous Thromboembolism

3.1 Introduction:

The introduction of the World Thrombosis day on October 13, 2014 highlights the disease burden associated with venous thromboembolism. The disease is currently a major public health issue associated with adverse outcomes and increased health care costs. Longer life expectancy and growing populations have led to increased disease prevalence. Increased prevalence is also associated with current lifestyle, sedentary work, obesity epidemic and the increased complexity of surgical and medical interventions.

The disease is multifactorial and involves interaction between acquired, inherited predispositions and environmental exposures. Diagnosis, using compression ultrasonography for detection of deep vein thrombosis (DVT) and computerized tomography (CT) for confirmation of pulmonary embolism (PE) can be easily established, but the speed and precision of diagnosis still need to improve. The available anticoagulant therapies once VTE has been diagnosed are becoming increasingly safer and convenient for the majority of patients. The non-vitamin K antagonist oral anticoagulants (NOACs) are increasingly becoming the standard of care for a large population of patients with VTE.

The estimated average annual incidence rate of overall VTE among Europeans ranges from 104 to 183 per 100,000 person-years. The incidence rates PE ± DVT and for leg DVT alone range from 29-78 and 45-117 per 100,000 person years respectively. (136-140) In a systemic review of the literature by Raskob et al in 2014, the annual incidence of VTE in countries such as Western Europe, North America, Australia and Southern Latin America ranges from 0.76 to 2.69 per 1000 individuals in the population. (141) In a study by Jha and et al it is reported that an estimated 3.9 million cases of hospitalizations associated with VTE occurred during one year among 1.1 billion citizens of high income countries (3.5 per 1000 population) and 6.0 million cases among 5.5 billion citizens of low and middle income

countries. VTE is therefore a common condition globally across different income regions. (142)

Data on trends in VTE over time are limited but overall VTE incidence either remained relatively stable or increased for the period 1981-2000 with a significant increase from 2000-2009. The more recent increase in incidence probably reflects increased utilization of objective imaging and improved image resolution. (143) Data on the number of VTE-associated deaths is also limited. Outcomes are more adverse following PE versus DVT (18 times higher risk of early death for PE versus DVT alone). For one quarter of patients with PE the initial clinical presentation will be sudden death and PE is an independent predictor of reduced survival for up to 3 months after initial occurrence. Increased age, male sex, lower body mass index (BMI), hospitalization, active malignancy and other comorbidities are independent predictors of reduced early survival. (144-147) One study using an incidence-based model suggests that in the United States approximately 300,000 per year are attributed to VTE (148) and a similar study reports 534,454 deaths related to VTE in the European Union in 2004.(149) Note that these are probably underestimates, as PE is often classified as sudden cardiac death or it might be reported as a contributing rather than primary cause in patients with cancer and other multiple comorbidities. VTE was the highest ranked cause of Disability Adjusted Life Years (DALYs) overall among seven major causes of hospital-associated adverse events in the study by Jha et al (142) In a review by Heit et al in 2016 on VTE-attributable costs it is reported that the adjusted mean predicted costs were 2.5 fold higher for patients with VTE related to current or recent hospitalization for acute medical illness compared to hospitalized controls matched on active cancer status from the VTE event date to 5 years post index. (150)

3.2 Risk factors for venous thromboembolism

Thrombus formation according to the classical knowledge requires Virchow's triad (151) of (1) Slowing down of blood flow- stasis (2) Hypercoagulability of the blood (3) Vessel wall damage. Venous thrombosis is mostly dependent on increased coagulability and venous

stasis rather than venous wall damage compared arterial thrombosis (the exception being for patients with indwelling catheters). The most common site of DVT initiation is the valve pocket sinuses of the calf veins due to the long periods of blood stasis and hypoxia. (152) Hypoxia and inflammation activate the endothelium to express adhesion receptors, endothelial selectin, VWF, P-selectin, and facilitates binding of leukocytes, platelets and microparticles and leads to increased TF expression, downregulation of TM and an overall shift of the hemostatic balance towards a hypercoagulable state. (153) Data from animal models has shown that platelets contribute to DVT propagation by binding to leukocytes and promoting secondary leukocyte recruitment at the thrombus site and that neutrophils amplify DVT development by forming neutrophil extracellular DNA traps (NETs) which trigger factor XII-dependent coagulation via the contact pathway and induce aggregation and activation of platelets. (154)

Risk factors for VTE include acquired risk factors and hereditary disorders of hemostasis. (table 10). (155-158) (159) (160) More than one acquired risk factor is often present and acquired risk factors often coexist with inherited ones. (158) Based on a population-based study on the incidence of VTE in 1999 the six most prevalent medical characteristics of patients with VTE were: more than 48 hours of immobility in the preceding month (45%), hospital admission in the past 3 months 39%), surgery in the past 3 months (34%), malignancy in the past three months (34%) infection in the past 3 months (34%) and current hospitalization (26%). (140) In a cross-over study which studied triggering events during the 90 day period prior to 399 hospitalizations for VTE compared to exposure which did not result in hospitalization for VTE

The incidence for hospitalized patients increases by almost 100 fold compared to residents in the community. (161) Hospitalization for medical illness or for surgery account for almost equal proportion of VTE. Among surgical patients VTE risk is further stratified based on age, type of surgery, presence of active cancer. Neurosurgery, major orthopedic surgery of the leg, thoracic, abdominal or pelvic surgery for cancer, renal transplantation, and cardiovascular surgery are high VTE risk surgical procedures. (162, 163) Similarly the risk in acute medical illness is further stratified based on age, obesity, previous VTE,

thrombophilia, cancer, recent trauma or surgery, CCF, prolonged immobilization, acute infection, central venous catheter, rheumatologic disorder, hormone therapy, acute myocardial infarction or stroke, white cell count and platelet count. (164-167). Active cancer accounts or almost 20% of all incident VTE occurring in the community and is higher in patients with brain, pancreas, ovary, colon, stomach, kidney and bone cancer and in patients with distant metastasis. Immunosuppressive and cytotoxic chemotherapy is associated with even higher risks particularly, thalidomide, lenalidomide and tamoxifen. (168-172)

3.2.1 Acquired risk factors:

Often patients with an episode of VTE have more than one acquired risk factor. Venous stasis due to immobilization is a very important risk factor for venous thrombosis. (173) Previous thromboembolism is a major risk factor for recurrent VTE which confers a relative risk of 7.9 approximately. One prospective cohort study reported a risk of recurrence of 18, 25 and 30 percent at 2,5, and 8 years. (174, 175) The risk of recurrence depends on other patient-specific risk factors and PE versus DVT confers a higher risk of recurrence. Risk of thrombosis increases post-surgery and particularly orthopedic, major vascular, neurosurgery, major abdominal and cancer surgery. (162) Surgical procedures are stratified into low, moderate and high risk according to the 2012 American College of Chest Physicians (ACCP). (176) All forms of major injury increase risk of VTE and the exact mechanisms are not understood but probably relate to immobility, reduced venous blood flow, decreased fibrinolysis, increased TF release and reduced production of endogenous anticoagulants. (177) Obesity and increased body mass index (BMI) are also significant risk factors. One study found a hazard ratio of 2.7 for a body mass index >40 for a VTE episode. (178) Many risk factors for VTE such as immobility, comorbidities, malignancy and others correlate to age but few studies adequately address these confounding variables.

The incidence of postoperative VTE in women on high dose estrogen therapy or full dose estrogen containing oral contraceptives are very high. Oral contraceptive use is the most important cause of thrombosis in young women, which is highest during the first

months of treated initiation, is unaffected by duration of use and returns to baseline risk 1-3 months following treatment cessation. (179) Oestrogen therapy increases the levels of coagulation factors II, VII, VIII, IX and X and decreased levels of AT and tPA in the vessel wall. The risk increases further in older and obese women and those with past VTE history. At least 6 studies have detected a relationship between smoking and VTE with relative risk ranging from 1.3-3.3. (180) (180) Women who smoke and receive oral contraceptives had a 8.8 fold higher risk than non-smoking women who don't use oral contraceptives. Pregnancy is also a risk factor for VTE and age-adjusted incidence of VTE ranges from 5-50 times higher in pregnant versus non-pregnant women. (181, 182) The risk is probably linked to obstruction of venous return and the hypercoagulable state associated with pregnancy. Other drugs linked to venous thromboembolism are testosterone, tamoxifen, Bevacizumab and glucocorticoids are associated also with increased VTE risk.

In states of inflammation there is an upregulation of coagulation factors and anticoagulant pathways are downregulated. Diseases with a particularly high risk are inflammatory bowel disease, Behcets disease, systemic tuberculosis, SLE and diabetes and chronic liver disease. In patients with end stage renal disease receiving dialysis VTE risk and particularly PE is also increased. (183) The mechanisms underlying the increased risk reported by some studies in non-dialysis patients are unknown but may be linked to elevated levels of factor VIII and VWF. (184) VTE prevalence post-renal transplantation ranges from 5-8% and nephrotic syndrome is also a significant risk factor. (185) (186) Potential mechanisms include reduced AT levels, increased protein C and S levels and platelet hyperreactivity. (187, 188)

The longitudinal investigation of Thromboembolism Etiology combined information from two prospective cohort studies, the Atherosclerosis Risk in Communities (ARIC) and the Cardiovascular Health Study (CHS) to investigate the relationship between risk factors for arterial disease and occurrence of VTE. (178, 189) Obesity, increased age, male sex, black ethnicity and diabetes were linked to increased VTE risk and hypertension, dyslipidemia, physical inactivity, smoking and alcohol consumption were not. Following arterial cardiovascular events there is an short-term increased VTE risk and one study reported a

4.22 risk following myocardial infarction and 4.41 post stroke. (190) Heart failure is a hypercoagulable state that can lead to intracardiac thrombi due to reduced ventricular left function and atrial fibrillation whereas the risk of DVT is highest in right heart failure due to peripheral edema.

The incidence of thrombosis in blood disorders that cause increased viscosity, thrombocytosis, altered platelet membrane receptors and responses are associated with high VTE risk. Myeloproliferative neoplasms (MPNs) such as polycythemia vera and essential thrombocythemia confer the highest risk for VTE among blood disorders. In a Swedish population-based study, compared with the control population, the incidence venous thromboses in patients with MPNs increased 10fold in the first three months after diagnosis and decreased in following years. (191) Waldenstrom's macroglobulinemia (WM) and Multiple myeloma cause hypergammaglobulinaemia and increased blood viscosity are also associated with higher VTE rates. Patients with WM may also develop IgM-related protein-losing enteropathy which may cause loss of anticoagulant proteins. Increased whole blood viscosity is also seen with white blood counts >100,000/microL seen in myeloid and monocytic leukemias.

The antiphospholipid antibody syndrome (APS) is characterized by the presence of persistent antiphospholipid antibodies and occurrence of thrombosis or recurrent miscarriage. It should be suspected in the presence of one or more unexplained events of VTE (or arterial thrombotic events) or recurrent miscarriages. It is primary condition but can also occur in the context of other conditions such as SLE and other autoimmune disorders.(192)

Patients with cancer have a hypercoagulable state due to the production of substances with procoagulant activity. Clinical VTE occurs in approximately 15% of such patients, approximately 20% of patients with symptomatic DVT have a known active malignancy. (193) (194, 195) The risk is highest during initial diagnosis, hospitalization, chemotherapy onset, disease progression and increases with increased disease stage and central venous catheter insertion. (196) Certain drugs may also increase the risk of VTE (high dose

dexamethasone, immunomodulatory agents such as lenalidomide and multiagent chemotherapy). VTE also has been reported by some as an adverse prognostic factor for patients with malignancy compared to diagnosis and characteristic matched patients with no VTE event. Patients with ovarian, brain and pancreatic cancer have a particularly increased risk of thrombosis but there is increased risk associated with all cancers.

Related to coagulation abnormality	• Hereditary hemostatic disorders: Factor V Leiden Prothrombin G20210A variant Protein C deficiency Antithrombin deficiency Protein S deficiency Abnormal fibrinogen Abnormal plasminogen • Hereditary or acquired hemostatic disorders Raised plasma levels of factor VII, VIII, IX or XI Raised plasma levels of fibrinogen Raised plasma levels of homocysteine Glycosylceramide deficiency Coagulation factor IX concentrates Lupus anticoagulant • Oestrogen therapy (HRT or contraceptive) • Heparin induced thrombocytopenia • Pregnancy and puerperium • Surgery especially abdominal and hip • Major trauma • Malignancy • Acute kidney injury • Myocardial infarction • Thrombocythemia • Immune thrombocytopenic purpura • Chronic autoimmune and inflammatory disorders (Systemic lupus erythematosus, sickle cell disease, Inflammatory bowel disease, psoriasis, vasculitis, celiac disease) • Infectious diseases (Tuberculosis, HIV, HCV)
Related to stasis	• Cardiac failure • Stroke • Prolonged immobility • Pelvic obstruction • Nephrotic syndrome

	• Dehydration • Hyperviscosity, polycythemia • Varicose veins • Central venous catheter insertion • Total parenteral nutrition • Chronic autoimmune and inflammatory disorders (Systemic lupus erythematosus, sickle cell disease, Inflammatory bowel disease, psoriasis, vasculitis, celiac disease)
Related to unknown factors	• Age • Obesity • Sepsis • Paroxysmal nocturnal hemoglobinuria • Behcets disease

Table 10: Summary of acquired and hereditary risk factors for venous thromboembolic disease

Gerotziafas et al have summarized in a review the most recently described risk factors for VTE in hospitalized medical patients. (197)

3.2.2 Hereditary disorders of hemostasis:

There are also a number of hereditary disorders of hemostasis which should be mostly suspected in young patients who have an event. (198) The most common causes of primary of hereditary thrombophilia are the factor V Leiden mutation and the prothrombin gene mutation which account together for 50-60% of cases. Defects in protein S, protein C and antithrombin account for the remaining cases. (199) The incidence of factor V Leiden (activated protein C resistance) is approximately 20-40%, it is caused by a genetic polymorphism in the factor V gene which makes it susceptible to cleavage by APC (the factor V Leiden mutation) and can be easily screened for by polymerase chain reaction (PCR). Heterozygotes for factor V Leiden are at a 5-8 fold increased risk of thrombosis compared to the general population and homozygotes are at a 30-140 fold risk for VTE compared to the population and heterozygotes at a 5-8fold risk. (200). The inheritance of antithrombin deficiency is autosomal dominant and leads to recurrent venous thrombosis and occasionally arterial thrombi. Many molecular variants of antithrombin have been categorized and are associated with varying degrees of thrombosis. Other hereditary disorders of hemostasis are protein C deficiency with autosomal dominant inheritance and

variable penetrance, protein S deficiency also autosomal dominant inheritance, prothrombin allele G20210A mutation which is a variant (prevalent in 2-3% of the population) that leads to increased plasma prothrombin levels and increases thrombotic risk by at least 2fold and hyperhomocysteinemia with higher levels of homocysteine which may be genetic or acquired and is associated with increased risk of both venous and arterial thrombosis.

3.3 Diagnostic modalities and algorithms:

The current diagnostic approach for patients with suspected VTE is currently based on algorithms that integrate clinical, laboratory data and imaging tests. (201) The Well's rule for estimated the clinical probability of DVT was first published in 1997 and combined five signs suggestive of DVT, three 2 factors and one negative variable to yield the probability score for DVT. The modified Well's risk score includes also the presence of previously documented DVT. (table 11) (202) Subsequently a Well's score for PE was developed. (203) (table 12) The Geneva score for PE is also a well-known pre-test clinical probability score for PE. (204) These scores have been developed and validated in the outpatient setting and they cannot be safely used to rule out VTE in the hospital setting.

D-dimer, the product of cross linked fibrin degradation which can be measured in nearly all laboratories has a high negative predictive value and accuracy in safely ruling out clinically suspected VTE.(205) Recently age-adjusted cut-offs for D-dimer have been validated and shown to improve the accuracy of the test. (206) Compression ultrasonography (CUS) is now the most commonly used imaging test as it is accurate, simple, reproducible and relatively inexpensive. CT is an important diagnostic tool for pelvic or inferior vena cava DVT while MRI also has high accuracy in preliminary studies. Ventilation/perfusion scan is highly sensitive for suspected PE but not very specific as there are many non-conclusive results and it is used only when there is a contraindication (pregnancy, renal insufficiency, allergy) to performing CT pulmonary angiography (CTPA) which is currently the first-line imaging test. Currently there are concerns about the overuse

of CTPA which has become readily available in emergency departments and inappropriate patient exposure to radiation. (207)

Components	Points
Active cancer (including treatment or within previous 6 months or palliative)	1
Paralysis, paresis or recent plaster immobilization of the lower extremities	1
Previous history of DVT	1
Recently bedridden for more than 3 days or major surgery within 4 weeks	1
Pitting edema	1
Entire leg swollen	1
Localized tenderness along distribution of the deep venous system	1
Calf swelling by more than 3 cm compared with asymptomatic leg	1
Collateral superficial veins (non-varicose)	1
Alternative diagnosis as likely or greater than that of DVT	-2
DVT unlikely	≤1
DVT likely	>1

Table 11: Well's score for suspected Deep vein thrombosis

Components	Points
Signs or symptoms of DVT	3
Alternative diagnosis less likely than PE	3
Heart rate >100/bmp	1.5
Immobilization or surgery in previous 4 weeks	1.5
Previous history of DVT or PE	1
Haemoptysis	1
Active cancer	1
PE unlikely	≤4
PE likely	>4

Table 12: Well's score for suspected Pulmonary embolism

3.4 Venous thromboembolism prophylaxis:

The first attempt to prevent thrombosis data back to 1899 when K.G Lennader, a Swedish surgeon, pointed out the benefit of hydration to maintain adequate blood circulation together with the elevation of legs with mild elastic compression. (207) Several other surgeons from the same era also discussed the importance of post-surgical early mobilization. This strategy gained more importance almost 50 years later when heparin had become available for clinical use. The first ones to describe its effectiveness in thrombosis prevention after surgery and trauma in 1939 were Cradord in Sweden and Murray in Canada. (207)

3.4.1. Available agents for thromboprophylaxis

The prototype vitamin K antagonist (VKA) was named dicoumarol and was reported in 1945 as useful in VTE prophylaxis after surgery in 148 patients. The first controlled trial on VTE prevention with acute fatal PE was published in UK in 1959 by Sevitt and Gallagher which compared the vitamin K antagonist phenindione with control in 150 patients with fractured femur. It was later suggested that heparin used in much smaller amounts could be effective for the prevention of VTE. V.V. Kakkar was the pioneer of clinical trials with low-dose heparin post-surgery. In a study of 53 patients post hernia surgery the incidence of VTE reduced from 26% to 4%. (208) The addition to low-dose heparin of dihydroergotamine, which improves venous emptying of pooled blood improved the prophylactic effect. Low molecular weight heparin (LMWH) was introduced in after results from a randomized clinical trial in 1982 which compared LMWH once versus twice daily after major abdominal surgery and showed the once daily injection to be effective and safe. (209) LMWH has longer half-life and better subcutaneous absorption as well as less binding to plasma proteins and platelets and unfractionated heparin and can be injected once daily versus 2-3 times/ day for unfractionated heparin. The pentasaccharide fondaparinux, the smallest entity of heparin that still binds to antithrombin was studied for multiple indications during the first decade of this millennium. Its use is limited by the triple cost it has compared to LMWH. It has however demonstrated a favorable benefit/risk ratio compared to LMWH in

acute coronary syndromes by reducing the risk of major bleeding and can also be used in patients with heparin induced thrombocytopenia. (210)

Orally available anticoagulants with a direct inhibiting effect on factor Xa or on thrombin have undergone extensive clinical trial assessment in major orthopedic surgery (211), for medically ill patients (212) (213) and to a lesser extent for patients with cancer.(214) The direct oral anticoagulants (DOACs) or Non-Vitamin K Oral anticoagulants (NOACs) including the direct factor IIa inhibitor dabigatran and the factor Xa inhibitors apixaban, rivaroxaban and edoxaban are being investigated for use in cancer patients. They have become increasingly favorable given their user-friendly route of administration, lack of need for monitoring at standard doses and reduced risk for food-drug interactions. They have received regulatory approval for treatment of acute VTE in general population but there is a paucity of data on the efficacy and safety of these drugs in cancer patients. None of the DOACs however are currently licensed for prophylaxis of thrombosis in cancer patients and there are limited data available for this population. Interestingly, they show comparable efficacy and improved safety compared to warfarin

The current indicated treatment for high-risk or massive PE is intravenous thrombolysis. Beyond the emergency situation however, given the risk of major bleeding, for normotensive patients with intermediate- or low-risk PE thrombolysis is not recommended (this recommendation is based on results from the PEITHO trial). (215) Emerging approaches to reperfusion treatment might allow comparable efficacy to intravenous thrombolysis without the associated risks of bleeding. Reduced-dose systemic thrombolysis and catheter-directed ultrasound-assisted low dose thrombolysis are being studied as potential alternatives. (207)

Parenteral anticoagulation was for many decades the mainstay of VTE treatment and prophylaxis. Initially unfractionated heparin was used, then Low molecular weight heparins (LMWH) or the synthetic pentasaccaride fonduparinux and vitamin K agonists (VKA). The use of these agents is still included in current recommendations but in the last decade two classes of Non-Vitamin K oral anticoagulants (NOACs) were approved for treatment and

prophylaxis of acute VTE; three direct factor Xa inhibitors (rivaroxaban, apixaban, edoxaban), and one direct thrombin inhibitor (dabigatran etexilate). Phase 3 clinical trials have demonstrated non-inferiority with respect to efficacy and a better safety profile (216) (217) and NOACs are therefore increasingly becoming the standard of care in anticoagulation for VTE.

Current consensus is to treat for a minimum of 3 months for a first episode of VTE but to extend beyond this time based on a case-by-case basis. There is uncertainty to this data regarding the effect and relative weight of individual parameters and predictors of recurrence risk or the use of bleeding scores in the assessment of the risk-to-benefit ratio of extending anticoagulation. (207)

3.4.2. VTE prophylaxis guidelines:

The American Society of Hematology published in 2018 guidelines for the prophylaxis of VTE in the hospitalized and non-hospitalized patient. (218)

- In acutely ill medical patients the ASH guidelines suggest the use of UFH, LMWH or fondaparinux rather than no parenteral anticoagulant and among these recommendation to use LMWH or fondaparinux rather than UFH.
- Critically ill medical patients: recommendation using UFH or LMWH over no UFH or LMWH and LMWH over UFH.
- In acutely ill medical patients the ASH guidelines recommend using LMWH over DOACs as VTE prophylaxis and only for the duration of hospitalization.
- Chronically ill medical patients or nursing home patients: recommendation not to use VTE prophylaxis in these patients.
- Medical out-patients with minor risk factors: no VTE prophylaxis use is recommended.
- Long-distance travelers: In people who are at substantially increased VTE risk (eg, recent surgery, prior history of VTE, postpartum women, active malignancy, or$2 risk factors, including combinations of the above with hormone replacement therapy, obesity, or

pregnancy), the ASH guideline panel suggests using graduated compression stockings or prophylactic LMWH for long-distance (>4hours) travel.

Given the paradigm shift of VTE risk assessment on an individual basis risk-assessment models (RAMs) have been developed for medical inpatients and the two most extensively studied and externally validated are the Padua score (219) and the IMPROVE score. (166) (table 13)

Risk assessment model (RAM)	Points
Padua VTE RAM score: ≥ 4 indicates high risk of VTE	
Reduced mobility	3
Active cancer	3
Previous VTE (excluding superficial thrombophlebitis)	3
Known thrombophilic condition	3
Recent trauma and/or surgery (<1 month)	2
Elderly age (>70years)	1
Cardiac and/or respiratory failure	1
Acute myocardial infarction or ischemic stroke	1
Ongoing hormonal treatment	1
Obesity (Body mass index >30)	1
Acute infection and/or rheumatologic disorder	1
IMRPOVE VTE RAM score: score ≥ 2 indicates high risk	
Previous VTE	3
Known thrombophilia	2
Lower limb paralysis	2
Active cancer	2
Immobilization ≥ 7 days	1
Intensive care unit/ Coronary care unit stay	1
Age > 60 years old	1
IMPROVE BLEEDING RAM: score ≥ 7 indicates high bleeding risk	
Renal failure (GFR 30-59 vs ≥ 60 ml/min/m^2)	1
Male versus female	1
Age 40-80 versus <40 years old	1.5
Current cancer	2
Rheumatic disease	2
Central venous catheter	2
Intensive care unit/ Coronary care unit stay	2.5
Renal failure (GFR < 30 vs > 60 ml/min/m^2)	2.5
Hepatic failure (INR >1.5)	2.5
Age ≥ 80 vs <40 years old	3.5
Platelet count (< 50 x 10^9)	4.0
Bleeding in 3 months prior to admission	4.0
Active gastrointestinal ulcer	4.5

Table 13: Currently used Risk assessment models (RAM) for the risk of thrombosis in medical patients: CI, confidence interval; CCU, Coronary Care Unit; GFR, glomerular filtration rate; ICU, Intensive Care Unit; INR, international normalized ratio. Padua risk score: Interpretation: among at-risk patients (Padua score ≥4), the reduction in VTE appears to outweigh the increased risk of bleeding with pharmacologic prophylaxis. Risk level: score of 0 or 1 low risk, score of 2 or 3 moderate risk; score ≥ 4 high risk. For scores > 2, VTE prophylaxis is indicated.

4.0 Venous thromboembolism and Cancer

4.1 Introduction:

Amand Trousseau is often credited as the first to describe the relationship between cancer and VTE but is was actually Jean-Baptiste Bouillaud who already in 1823 reported a case of deep venous thrombosis in a cancer patient. (220) Malignancy is a major risk factor for thromboembolic disease and approximately 20% of patients with symptomatic DVT will have an underlying malignancy. The risk of VTE is 4 to 7 fold increased in cancer patients compared to healthy individuals (221) (222) and about 15% of cancer patients will experience a VTE event.

Survival rates are significantly lower and prognosis significantly worse in cancer patients with VTE relative to those without and VTE is the second cause of death, after cancer itself in this population. (223) (224) The economic burden associated with VTE in cancer is also high as diagnosis and management of thrombotic events interrupt essential therapies, carry risks of serious bleeding complications, often require hospital admissions and lead to higher risk of VTE recurrence. Healthcare costs are approximately 40-50% higher in cancer patients with VTE compared to cancer patients without VTE. (225, 226)

The most common localization of CAT is deep vein thrombosis (DVT), PE and central-venous catheter associated thrombosis. Thrombosis in the portal vein, splenic vein, mesenteric vein and renal veins is also frequent in patients with primary hepatic, pancreatic, kidney and suprarenal cancers. Asymptomatic or incidental DVT and/or PE is also relatively common in cancer patients. A thrombus in the pulmonary artery is accidentally detected in approximately 3% of patients. (227, 228)

4.2 **Cancer associated VTE risk factors**

Risk factors for VTE can be divided into patient-related, cancer-related and treatment related.

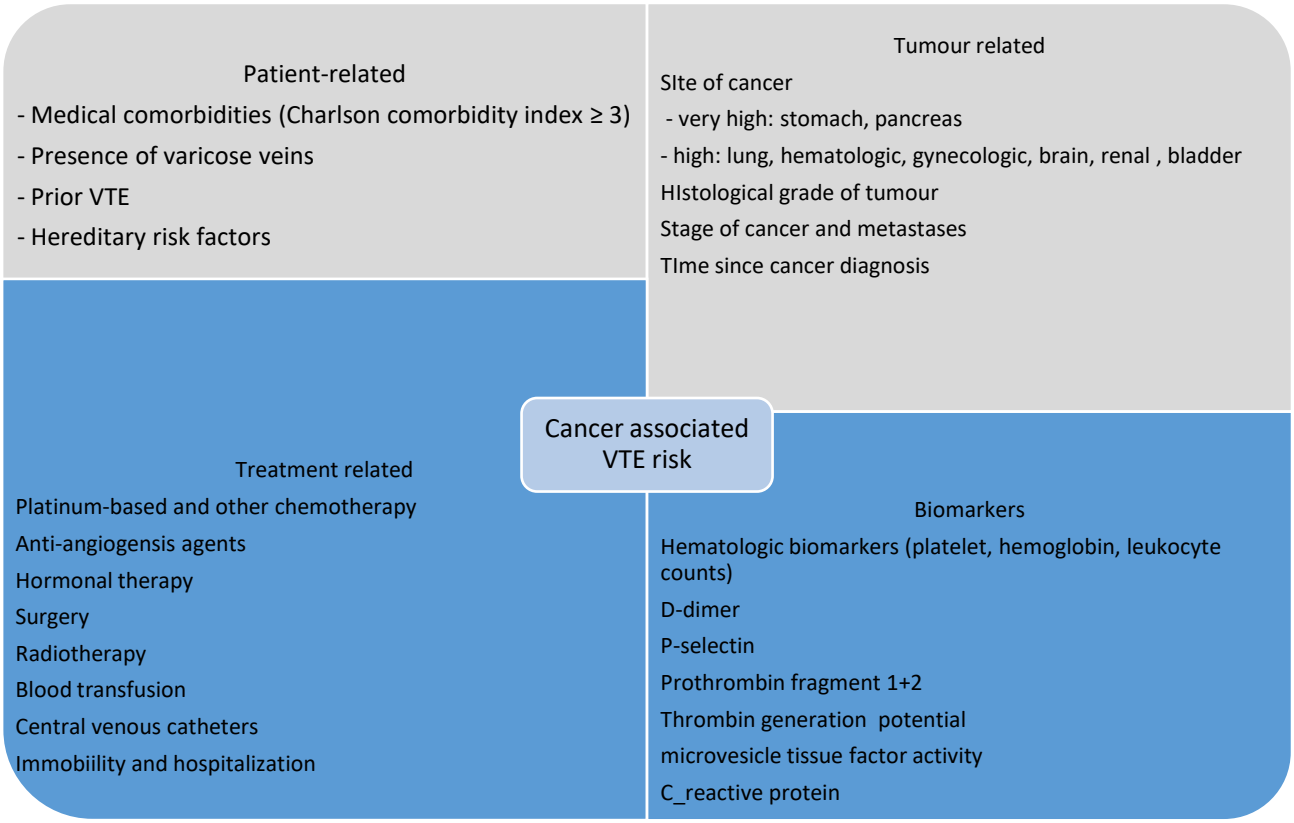


Figure 1: Cancer associated VTE risk factors

Patient related risk factors

Patient -related include standard risk factors for VTE such as advanced age, obesity, black race, immobilization, prior VTE history, infection, anemia, renal disease and other medical comorbidities. Family history of VTE and hereditary thrombophilia are also important risk factors.

Treatment related risk factors

Anti-cancer treatments like chemotherapy, hormonal therapy, anti-angiogenic therapy and erythropoiesis stimulating agents, blood transfusions and central venous lines all contribute to an increased risk. Chemotherapy is an independent risk factor for VTE. (229) In a retrospective record-linkage cohort study chemotherapy was associated with a 2fold – 6fold increase of VTE compared with the general population. (169) There are various mechanisms through which chemotherapy induces a prothrombotic state. (230) It causes direct tissue toxicity, increases the levels of procoagulant molecules and reduces the levels of

endogenous anticoagulants, induces tumor and endothelial cell apoptosis and cytokine release (leading to increased expression and TF activity), induces platelet activation and expression of monocyte-macrophage TF. Immunomodulatory agents (IMiDs) used in patients with multiple myeloma (MM) such as lenalidomide and thalidomide are associated with high VTE risks particularly when combined with high doses of dexamethasone or multiagent chemotherapy as it will be extensively discussed below. (231)

Tumor related risk factors

The type of cancer, high tumor grade, disease stage and metastasis and cancer related procedures such as surgery and chemotherapy are also important CAT risk factors (168). Recent studies have shown that malignant brain tumours, haematological malignancies and adenocarcinomas of the pancreas, uterus, ovary, stomach and kidney confer the highest risk of VTE. (232) Rates are higher among those with advanced metastatic cancers and high histological grades. In the Vienna CATS study the risk of developing VTE was twice as high in patients with high grade tumors (G3 and G4) compared to those with low grade. (233) The risk of CAT is also highest in the initial period following the cancer diagnosis. (169)

Cancer-driven events seem to specifically promote a hypercoagulable state which includes increased activation of procoagulant factors and inhibition of anticoagulant mechanisms, impaired fibrinolysis, production and secretion of procoagulant substances and proinflammatory cytokines, increased platelet aggregation and adhesive interactions among tumor cells, endothelium and blood cells. (234) There are reports of oncogene and tumor suppressor genes (induction of K-ras, loss or p53, loss of PTEN, activation of MET) (235) which can also promote hemostatic changes.

4.3 Biomarkers for Cancer associated thrombosis

In an attempt to identify more accurately patients with malignancy at high risk of thrombosis a number of groups have studied laboratory biomarkers that could help identify

those patients at risk. As stated by Strimbu and Tavel “The term biomarker refers to abroad subcategory of medical signs observed the patient that can be measured accurately and reproducibly”. It is therefore hypothesized that biomarkers of a prothrombotic state can be used to identify patients at increased risk for primary and recurrent VTE. (236)

The most studied biomarkers which have demonstrated some efficacy and have most consistently been associated with high risk of VTE in cancer patients are platelets, d-dimer, fibrinogen and P-selectin. (237) Additional biomarkers like thrombin generation (238), prothrombin fragments (239) and tissue factor microparticles (240) have shown promise but at the same time show significant limitations such as variable sensitivity and specificity depending on the type of tumour subtype. Other studied biomarkers include mean platelet volume, soluble VEGF, factor VIII, protein C, protein S, von Willebrand antigen, antithrombin, thrombin antithrombin complex, antiphospholipid antibody, plasminogen activator inhibitor, tissue factor pathway inhibitor and several variants associated with hypercoagulable states have been reviewed but no significant associations have consistently been demonstrated. (241)

Data to support any of the biomarkers discussed here in routine clinical decision-making are currently lacking, but additional investigation in clinical studies, ideally in combination with clinical factors known to be associated with increased thrombotic risk, is warranted. One of the largest studies that have reported an association between VTE and d-dimers was performed by Libourel et al and included 404 patients with acute myeloma leukaemia (AML).

Hazard ratio (HR) for VTE among patients with d-dimer 0.5–4.0mg/L and >4.0mg/L vs ≤ 0.5mg/L were 5.58 (95% CI 0.62–49.97) and 32.05 (95% CI 3.58–286.83) (p = 0.002) respectively.(242) In a subanalysis of data from the Vienna Cancer and Thrombosis Study out of 111 patients with haematological malignancies 8 patients had a VTE and elevated d-dimer levels (>1.4 mg/L, the 75th percentile) were found to be positively associated with an increased risk of VTE (HR 1.8, IQR 1.0–3.2) among all patients enrolled in this study. (239) In the study by Libourel et al the HR for venous thrombosis with fibrinogen <1.0g/L was 12.38 (95% CI 1.54-99.18) in the cohort of AML patients. Other studies have not found data to

support this finding. (243) Among studies which examined the role of antithrombin and VTE risk all (243) (242) but one found no association. The only study that has demonstrated a positive association was performed in 30 children undergoing therapy for ALL. Two separate studies have demonstrated a relationship between thrombin generation and VTE. Ay et al used data from the Vienna CATS cohort and out of the 152 patients 10 had a VTE among which median peak thrombin generation was found to be significantly higher compared to those who did not experience VTE.(238) In another study in 56 children by B lineage ALL patients who experienced thrombosis had consistently higher endogenous thrombin potential (ETP) and peak values. (244)

Thrombocytosis has been established as a risk factor for VTE in cancer patients and it is therefore included in the Khorana risk assessment score for VTE prediction in ambulatory cancer patients (platelet >=350,000 μ/l). (245) In the Vienna CATs cohort P-selectin was also found to be higher among 4 patients who experienced VTE out of the 91 patients who had a measurement of this value. (246) This group demonstrated that elevated soluble P-selectin is associated with higher incidence of VTE in cancer patients in a prospective analysis of 687 patients with malignancies. P-selectin was shown to independently predict VTE after adjusting for age, gender and cancer treatments (HR 2.6, P=0.003). The same group showed that addition of soluble P-selectin to the Khorana risk improved its prediction power. (247) Platelet factor 3 (a chemotactic peptide produced by megakaryocytes and stored in alpha granules of platelets) has also been shown by some studies to be a biomarker of VTE risk. (248)

Prospective data have linked elevated levels of MPs with thrombosis occurrence (240) (249) (250) but other studies have failed to demonstrated that an increase in MPs is a predictive biomarker for future thrombosis (251, 252). (table 14) The discrepancy maybe related to variability of thrombotic risk with different malignancies or differences in methodologies and variables chosen for analysis. The most convincing data comes from a prospective study of serial MP TF-dependent procoagulant activity measurements in patients with pancreatic cancer as a significant correlation between increasing levels over time and thrombosis development is demonstrated. (253) The study by Campello et al in

2011 (254) mentioned previously demonstrated that cancer patients have higher levels of circulating MPs which could be one of the potential mechanisms underlying increased VTE risk. Other studies have also demonstrated that cancer patients with thrombosis have higher MP levels than cancer patients without thrombosis. (240, 255, 256). Data on a causal relationship between elevated MPs and thrombosis are inconclusive so far.

Patients	Without VTE/ with VTE	Follow up	Main results
Cancer without VTE at study entry (249)	43/5	6 months	Annexin V+MPs levels not predictive of VTE
Cancer without VTE (240)	60/5	1 year	TF+MP were higher in patients with VTE (OR 3.72 95% CI: 1.18-11.76)
Glioblastoma multiforme (257)	61/11	7 months	TF+MP levels above the 90th percentile were associated with a higher risk of VTE (RR: 4.17, 95% CI: 1.57-11.03)
Solid and hematological cancer (251)	728/53	2 years	PS+MP were not predictive of VTE
Cancer without VTE at study entry (249)	43/5	6 months	MP TF activity was higher in patients who developed VTE
Locally advanced or metastatic pancreatic cancer (253, 258)	11/2	Every 4 weeks for 20 years	MP TF activity higher in patients who developed a VTE
Pancreaticobiliary cancer (195)	117/52		MP-TF activity associated with VTE (OR 1.4, 95% CI 1.1-1.6)
Multiple myeloma before chemotherapy (252)	122/15	Not specified	No association
Cancer without VTE at study entry (251)	299/49	2 years	Borderline significant association between MP-TF activity and VTE

Table 14: Studies on the role of Microparticles (MP)levels and the risk of cancer associated Venous thromboembolism (VTE) occurrence. TF: tissue factor; PS: phosphatidylserine.

4.4 Risk assessment models for CAT

In order to select the patients at higher risk for VTE who will benefit from thromboprophylaxis there is a need for development of accurate prediction models. As

more is understood about the CAT mechanisms these models will become more accurate. The development of risk assessment models (RAM) for thrombosis in cancer patients has been a popular area of research for some years. Several scores for predicting the risk in ambulatory outpatients with cancer have been developed. (239) (246) (259) (260, 261)

A number of discriminatory parameters (tumor entity, BMI, hemoglobin level, leukocyte count) and putative biomarkers (D-dimer, prothrombin garment 1+2, soluble P-selectin, TAT complexes and TF-bearing MPs) for VTE risk have been proposed and several have been incorporated into risk assessment scores, designed to aid in identification of those cancer patients at risk of thrombosis. However recent evaluation of current risk scores in a multinational prospective cohort study of patients with advanced solid cancers highlighted their poor discriminatory capacity (262) and as Ünlü and Versteeg discuss in their review recently, overall the accuracy of such tools is however low. (263) (264) The main limitations are that (i) while they perform well in large cohort studies they are unable to predict CAT at the individual level (ii) they are not developed for specific cancer types (iii) they underperform when used to predict risk of VTE recurrence and (iv) they poorly predict increased risk of mortality. The addition of variables that are classically associated with VTE such as platelet count, D-Dimer and P-selectin levels moderately improves their power but at the same time these plasma-derived biomarkers are sensitive to circumstances like inflammation, surgery and chemotherapy and great variability is introduced in their plasma concentration. (265)

Among these the Khorana score introduced in 2008 has been validated in large cohorts of patients with a variety of malignancies who are undergoing chemotherapy. (245) The Khorana score is calculated by assigning points for clinical parameters available for most patients (site of primary tumor, hematologic parameters and BMI). It was derived in a cohort of 2701 patients with cancer undergoing chemotherapy and validated in an independent cohort of 1365 patients. Patients were stratified into three risk groups to predict the development of VTE. The cumulative incidence of VTE at 2.5 months ranged from 0.3 percent to 6.7 percent in patients with the fewest and most risk factors, respectively.

Most studies of the Khorana score evaluate the performance of the score over 6 months, often corresponding to the duration of chemotherapy. (table 15) Since its first introduction it has been updated and validated a few times. The Vienna CATS group expanded the score by adding two biomarkers, D-dimers and soluble P-selectin. (247) The score was also validated in an independent study of 1415 patients with advanced malignancy enrolled in phase 1 chemotherapy trials and a modified version was used in an observational cohort study the Vienna Cancer and Thrombosis study. (247, 266). The modified score includes additional high risk tumor types (myeloma, brain, kidney) and the two additional laboratory values (D-dimer and P-selectin). Later, following the posthoc subgroup analysis of the PROTECHT trial, the original score was validated and modified to the "Protecht score" by the addition of exposure to specific chemotherapy agents. (267)

The CoTOn- ER2UPMC group has introduced the 4TS-COMPASSE RAM for identifying cancer patients eligible for antithrombotic prophylaxis but the model awaits validation. It includes the following variables: anthracycline or anti-hormonal therapy, time since cancer diagnosis, central venous catheter, cancer stage, presence of cardiovascular risk factors, recent hospitalization for acute medical illness, personal history of VTE, and the platelet count. (259) Other scores include the CONKO-004 score (268) and the ONKOTEV study (269). A 2017 cohort study attempted to compare efficacy of the different tools, but 70% of patients were enrolled up to 3 months after the start of therapy which nullifies the utility of the components of these scores that rely on baseline variables prior to the start of chemotherapy. (262)

More recently Pabinger et al used data from the Vienna Caner and Thrombosis Study (CATS) and selected prognostic variables for inclusion in the model. They reported on the development and validation of a tool that utilizes only two variables: type of cancer (low vs intermediate vs high vs very high tumor site risk) and D-dimer levels (continuous concentrations) with varying effects of the latter for different types of solid cancers. They show that the model outperforms previous clinical prediction scores and predicts patients at high risk of developing CAT. The tool is also available as a nomogram and an online

prediction tool. Further data is awaited to establish whether this new tool is also predictive of benefit from thromboprophylaxis. (270)

The Khonara score which was initially developed and internally validated in a cohort of ambulatory patients with solid tumor diagnoses initiating systemic chemotherapy followed for four cycles of therapy These results have been validated by multiple, independent, external validation studies. (271) The Khorana score has also been shown to predict a benefit for thromboprophylaxis in patients with a risk of 3 or higher in subgroup and/or pooled analyses. (267, 272) Two more recent trials on thromboprophylaxis were restricted to patients with a Khorana score of 2 or higher. (273) (274)

One meta-analysis determined the VTE risk in almost 35,000 ambulatory cancer patients over 6 month follow up and most were retrospectively analyzed to include a number of tumor types. Most patients had a score of 1 or 2 and the 6 month risk correlated with the score: those with a score of 0 had a 5% risk, those with a score of 1 or 2 a 6.6% risk and those with a score of 3 or greater had a 11% risk. (275) A retrospective analysis the cumulative incidence rates of VTE at six months were 1% for the lowest risk group (0 points) and 35% for the highest risk group ((≥5). Additional studies have confirmed higher incidence of VTE in those with abnormal coagulation studies, including elevated D-dimer levels, peak thrombin generation, prothrombin fragment 1+2, tissue factor and fibrinogen. (239) (238) (270) The most recently developed risk score, Tic-Onco also includes genetic risk factors and showed a positive predictive value of up to 37% improving the predictive values obtained by the Khorana score that correctly predicts VTE in 22% of the CAT patients. (276)

Khorana score				
Risk factor			Score	
Site of primary tumor				
	Very high risk (stomach, pancreas)		2	
	High risk (lung, lymphoma, gynecologic, bladder, testicular)		1	
	All other sites		0	
Pre-chemotherapy platelet count >=350.000/microL			1	
Hemoglobin level <10 g/dl or use of ESAs			1	
Pre-chemotherapy WBC >11,000/ microL			1	
BMI **≥ 35** kg/ m2			1	

Khorana score points	Derivation cohort VTE risk after 2.5 months (245)	Validation cohort VTE risk after 2.5 months (245)	Independent cohort VTE risk after 6 months (247)	Phase I trial VTE risk after 2 months (266)
0 (low)	0.8%	0.3%	1.5%	
1 to 2 (intermediate)	1.8%	2%	3.8% (1 point) 9.6% (2 points)	4.8%
≥3 (high)	7.1%	6.7%	17.7%	12.9%

Table 15: Khorana score

The Ottawa score aims to stratify the risk of VTE recurrence in cancer patients during the first 6 months. The original scores includes female sex, lung cancer and prior history of VTE assigning 1 point, breast cancer a negative point and cancer sage 1 two negative points and dichotomizes patients into low (score ≤ 0) or high risk (score ≥ 1) for VTE recurrence. The modified score assigns one point for female sex, lung cancer, prior VTE, a negative point for breast cancer or cancer stage I +II and classifies patients into low (score ≤−1), intermediate (score = 0), and high risk (score ≥ 1) for VTE recurrence.(277, 278)

4.5 Pathogenesis of the hypercoagulable state in cancer

The procoagulant state is the result of a multifactorial interplay as the tumor cell will express or exert procoagulant activity, at the same time the normal host coagulation cascade balance may drift towards the hypercoagulability side in response to the cancer and comorbid factors such as immobility, surgery, medication and infection increase the risk further and may determine whether an asymptomatic increase in coagulability will manifest itself clinically. (232, 279) The pathogenesis of VTE in cancer is multifactorial and rather unclear. The manifestation may range from asymptomatic abnormal coagulation tests to migratory superficial thrombophlebitis, idiopathic deep vein thrombosis, nonbacterial thrombotic endocarditis, disseminated intravascular coagulation, thrombotic microangiopathy and arterial thrombosis. Cancer cells exert prothrombotic effects on their microenvironment through direct and indirect mechanisms. Neoplastic cells will exert their prothrombotic effect directly via the release procoagulants directly (TF, cancer procoagulant) and indirectly via the release of cytokines and production of factor-X activating cysteine proteases, mucinous glycoproteins and circulating TF-MP.(280) A growing body of evidence also supports the idea that some genetic mechanisms responsible for malignant transformation including activation of oncogenes, such as RAS and PTEN, and inactivation of tumour suppressor genes, such as p53 and PTEN) drive the expression of genes controlling hemostasis. Thrombin and fibrin generation via the above mechanisms results in the fibrin scaffold which then potentially confers a selective advantage to cancer cells allowing for tumor anchorage and invasion. The relationship is therefore bidirectional. (281)

Cancer procoagulant activity and Cancer procoagulant (CP): Earlier studies have made a distinction between tissue-factor like procoagulant activity of tumors and cancer procoagulant activity.(282) CP is a calcium dependent cysteine protease that has been found in malignant and fetal tissue but not normally in differentiated tissue. CP activates factor X directly independent of the TF/factor VIIa complex. It has been reported to be present in extracts of cells from patients with acute promyelocytic leukemia, melanoma,

colon cancer, breast lung and kidney cancer. Currently however there is not enough data to support an important role of CP in the hypercoagulable state associated with malignancy.

Procoagulant activities of host tissues: In patients with malignancy normal cells are stimulated to express procoagulant activity and activation of hemostatic mechanisms. Among these activators is the cell adhesion molecule P-selectin found in the alpha granules of platelets and the Wiebel-Palade bodies of endothelial cells which increases the expression of TF on monocytes and endothelial cells. High P-selectin levels have been associated with increased VTE risk in cancer patients. (246) Monocytes may be activated by tumor-specific antigens and immune complexes that involve tumor antigens or directly by the cytokines secreted by other immune cells in response to tumor related antigens. Activation can lead to tissue factor production and production of other direct factor X activators.

Endothelial cells: Endothelial cells also become procoagulant under the action of cytokines such as tumor necrosis factor (TNF) and interleukin (IL)-1 which increase the expression of leukocyte adhesion molecules, platelet activating factor and tissue factor. TNF suppresses endothelial fibrinolytic activity and increases IL-1 production, downregulates thrombomodulin expression, diminishing the activity of anticoagulant protein C. In vitro TNF enhances the procoagulant and suppresses the anticoagulant properties of cultured endothelial cells. (283) It has not been determined whether TNF levels are consistently elevated in malignancy and data is contradictory.

Platelets: The complex relationship between cancer and platelets is bidirectional, platelets impact tumor behavior and vice versa. Platelets have emerged as important mediators in tumor development, malignant cell growth, angiogenesis and metastasis but there is also growing evidence that they have a role in promoting the hypercoagulable state of malignancy.

The presence of cancer results in numerical and functional changes in platelets and changes in their activation status both directly and indirectly. Thrombocytosis is observed in cancer patients and thrombocytopenia has also been shown to be associated with antimetastatic

effects in experimental models. The relationship between cancer and platelets has been acknowledged since the late 19th century when thrombocytosis was associated with solid tumor cells. Numerical changes in platelet counts provide useful prognostic information in cancer patients as thrombocytosis is associated with decreased survival in multiple cancers (lung, ovary, endometrium, rectum, brain, breast and more) suggesting that they contribute to cancer progression and metastasis. (220) Cancer induced or associated alterations in platelet number or function are associated with the prothrombotic tendency observed in cancer patients. Thrombocytosis is the only validated cancer associated VTE risk assessment score to date where patients are assigned one point when they have pre-chemotherapy counts over 350 x 10^9/L. (245)

Cancer-induced thrombocytosis:

Many studies have demonstrated that an elevated platelet account is associated with increased risk of cancer associated thrombosis. In the Vienna Cancer-associated thrombosis study (CATS), a prospective observational study of cancer patients, the one year cumulate VTE rate was 34.3% in patients with platelet count in the upper 5th percentile compared to 5.9% in all other patients and thrombocytosis (platelets >350,000 µl) was associated with a 2fold higher risk of VTE (HR 2.4, p=0.01). (284) The Khorana risk model, a validated tool for risk assessment of cancer associated thrombosis in ambulatory patients, includes thrombocytosis (platelets ≥350,000 /µl). (245) Thrombocytosis is also observed in infectious and inflammatory disease and it is hypothesized that these mechanisms may have common components. Cytokines stimulate megakaryocyte formation and platelet production and cancer frequently induces similar systemic effects with increased levels of pro-inflammatory cytokines. However in the prospective Vienna CATs cohort of patients with various cancers no association was found between plasma TPO levels and platelet count (284) suggesting that cytokine induced increased platelet production may not be the main mechanisms via which platelet counts are increased in patients with cancer. A possible explanation is increased clearance of TPO from the circulation by platelets. Alternatively TPO may be produced directly by cancer cells but evidence to support this hypothesis in vivo is scarce. The concept that cancer cells act as inducers of platelet activation and aggregation is well established and platelet activation stimulates cancer progression. Tumor cells induce

platelet aggregation which leads to a shield of aggregated platelets around circulating tumor cells (CTCs) protecting it from attacks by the immune system. CTCs have a short half-life as they are rapidly cleared from the circulation by the immune system of the host but when they are coated by activated platelets they are enveloped and shielded from immunological detection and attacks by natural killer cells. (285) The numerous platelet receptors may also aid the extravasation of the CTCs at distant sites by establishing firm attachment of the platelet-tumor cell aggregates to the endothelium. When the CTC-platelet complex is large enough it may even embolize the microvasculature of distant organs and support metastasis.

Direct cancer-induced platelet activation:

This process of cancer activation and aggregation is called tumor-cell induced platelet aggregation (TCIPA). It has been demonstrated in vitro from multiple cancer cell lines and the process is tumor type dependent but the mechanisms has not been fully elucidated. (220) One proposed mechanism is via the activation of the transmembrane protein podoplanin on various tumor cell lines which binds to the C-type lectin-like receptor (CLEC02) on the platelet surface triggering platelet activation. Other proteins implicated are tumor-derived matrix metalloproteinase (MMP-2) and cathepsin B. Tissue factor generated by cancer cells can also activate platelets. Tumor cells also shed small extracellular vesicles that expose the transmembrane protein tissue factor (TF) which is the initiator of the extrinsic coagulation cascade.(286) Abundant tissue factor leads to thrombin activation the most potent platelet activator.

Indirect cancer-induced platelet activation:

Cancer cells shed coagulant extracellular vesicles (EV) including exosomes and microparticles which are able to initiate thrombin generation. EV are small cell-derived membrane vesicles that bud off of activated cells such as endothelial cells, platelets and leukocytes but also cancer cells. They inherit transmembrane proteins from their parent cell and as many tumor cells overexpress TF, cancer cells release EV exposing TF. (287) High levels and high coagulant activity of TF-exposing EV have been associated with cancer associated VTE.(256) (240) EV most likely exhibit their coagulant activity by adhering to

activated platelets by binding to P-selectin glycoprotein ligand 1 to its counter receptor exposed on the surface of activated platelets.

Another indirect pathway is through cancer induced formation of neutrophil extracellular DNA traps (NETs) in a process called NETosis. (288) These are intravascular networks of DNA fibers released from neutrophil nuclei by a multistep programmed form of cell death (NETosis) that were first identified as a host defense mechanisms against pathogens. Platelets stimulate release of NETs and in turn NETS induce platelet adhesions, activation and aggregation in vitro. The platelet-NET interaction results in a vicious prothrombotic cycle, and there is emerging evidence these interactions play a role also in cancer and cancer-associated thrombosis. They also coat CTCs with platelets to aid in immune escape. (289, 290) Platelets are also activated by the increased formation of extracellular traps in the presence of tumor secreted granulocyte colony stimulating factor. The process of granule secretion by platelets is tightly regulated and secretion is selective. In vitro cancer cells seem to mediate selective ADP secretion from platelet dense bodies which is called tumor cell induced platelet secretion and may contribute to stabilization of angiogenesis in the tumor microenvironment and promote tumor growth.

Platelet activation in cancer patients ex vivo:

Markers of platelet activation like P-selectin, CD40 ligand, β-thromboglobulin are often elevated in cancer patients and these tend to be associated with advanced cancer suggesting either an effect of platelets on disease progression or increased platelet activation induced by higher stage tumors. (291) In a large cohort (687 patients with various malignancies), the Vienna group, showed that the risk of VTE was 2.6 times higher in cancer patients with P-selectin plasma levels in the upper quartile compared to those with lower levels but P-selectin is not specific for platelets but also exposed on activated endothelial cells the increased levels of P-selectin may not solely reflect platelet activation. (246) The same group showed that addition of soluble P-selectin to the Khorana risk score improved prediction. Several lines of evidence suggest also a role for platelets in tumorigenesis, both at early stages and in the process of metastasis. They play a role in the interaction between cancer cells and the stromal cellular component and in promoting distant organ

colonization. These effects are mediated either via direct interaction with target cells and/or release of mediators. Several lines of evidence show that the release of platelet microparticles (MP) is an important mechanism for caner promotion induced by activated platelets. Platelet derived MPs are rich in proteins and genetic material including microRNAs which can be delivered to other cells. Also interestingly cancer cells can acquire platelet-derived proteins from MPs – mimicry of cancer cells. This can then activate the coagulation cascade or the platelets and facilitate the hematogenous dissemination of cancer cells. (292-294) Mechanisms suggested for platelet activation in malignancy include tumor-induced thrombin generation, ADP production by tumor cells and increased levels of won Willebrand factor (vWF). (295)

Tissue factor :

TF is often considered the center of cancer-associated thrombosis as it plays a role in both tumor progression and VTE. There is upregulation of TF on the surface of the tumor cell and migration into the vascular lumen during vascular invasion and metastasis but also TF-positive inflammatory and stromal cells within the tumor microenvironment, upregulation tumor vasculature TF expression by angiogenic or activated ECs and the release of TF-MPs into the blood circulation. (256) (296) TF expression varies among different types of cancer and also increases with advanced cancer stage. Expression of TF by cancer cells is under the transcriptional control of oncogenes and tumor suppression genes such as members of the EGFR family, RAS, TP53 and PTEN. (232)

Pancreatic cancer cells do exhibit markedly high levels of TF expression which has been linked to procoagulant activity in vitro. The risk of VTE was increased 4fold in patients with high tumor TF expression when compared to those with low TF levels. (253, 258) In another relatively small cohort of patients with ovarian cancer TF expression showed a correlation with the incidence of thrombosis and D-dimer levels. (297) However not all studies have confirmed a link between TF and VTE and the contribution of TF-MPs to VTE risk may be highly variable between tumor types and cancer-type specific. Differences may be due to different levels of TF encryption and participation of TF in non-coagulant activities. A definite link between TF-MP and clinical VTE development has yet to be established and remains debated. (287)

TF-bearing microparticles (TFBP) have been studied using flow cytometry in patients with malignancy in a study by Zwicker et al in 2009 (240). It was shown that TFMP are detected in patients with advanced malignancy and are associated with VTE in a cohort of 96 cancer patients of different histologies. The link between TF-MP and VTE has been only established in pancreatic cancer patients in the clinical setting and no correlation has been demonstrated for brain, colorectal or lung cancer patients. (287)

Elevated microparticles and cancer associated thrombosis: Many studies have demonstrated high levels of circulating procoagulant MPs which may originate from various cell types. MPs most commonly detected in cancer patients include PMPs, monocyte-derived MPs (MDMPs) and endothelial-derived MPs (EDMPs). A lot of recent evidence supports the role of platelet MPs in cancer and metastasis and multidrug resistance in patients with chemotherapy. (298) Cancer associated MPs were first described in the plasma of patients with Hodgkin's disease and subsequently increases in the total "circulating" MPs and their procoagulant activity in these contexts have been reported in multiple cancer types. Patients with myeloproliferative neoplasms are at high risk of venous thromboembolism and studies have shown a significantly higher number of MP than controls most of them derived from platelets and endothelial cells. Thrombin generation assays shown shorter lag time, higher peak height, higher median velocity index in MP-rich plasma of MPN patients compared to control suggesting a role for MP in thrombogenesis in these patients. (299) Campello et al in 2011 measured the levels of endothelial MPs (EMPs), platelet MPs (PMPs) and Tissue Factor- bearing MPs (TF+MPs) in the plasma of 90 consecutive patients- cases (30 with VTE, 30 with active cancer and 30 with active cancer and acute VTE) and in a group of 90 healthy controls. Patients showed statistically significant higher (mean±SD) circulating EMPs and PMPs plasma levels (920±341 and 1221±413 MP/µL, respectively) than controls (299±102 and 495±241 MP/µL; pb0.005). Cancer patients with and without VTE showed higher (mean±SD) TF+MPs (927±415 MPs/µL) than controls (204±112 MPs/µL; pb0.001). The subgroup of cancer patients plus VTE showed statistically significant higher TF+MPs plasma levels (1019±656 MPs/µL) than cancer patients without VTE (755±391 MPs/µL, p=0.002). Multivariate analysis however failed to

show a significant association between elevated TF+MPs and VTE in cancer patients.(254) Hron et al (300) also demonstrated that the number of circulating MPs was increased about 2 times in patients with colorectal cancer compared to healthy controls. Tesselaar et al also demonstrated that patients with different types of cancer plus VTE had higher number of circulating MPs and particularly TF bearing MPs than cancer patients without VTE. (256, 301) Similarly Zwicker at al confirmed these findings. (240) There is also increasing data to support that the cancer cells themselves may be the source of the procoagulant MPs.

Heparanase:

Studies have shown a correlation between the activity of heparanase and the metastatic potential of cancer cells, it has been implicated in neovascularization, inflammation and autoimmunity and an upregulation has been demonstrated in multiple human tumors examined. (302, 303) More specifically increased levels have been demonstrated in colon, thyroid, pancreas, bladder, gastric, prostate and multiple myeloma, leukemia and lymphoma. (304) (305)It has been hypothesized that heparinase may act as a cofactor to TF as based on results from some in vitro studies it is over-expressed in some cancers and results in increased levels of TF.(306) In vitro studies have also shown that exogenous addition or overexpression of heparinase by transfected cells results in increase of TFPI from the cell surface and accumulation in the cell culture medium. These studies were supported by elevation of TFPI in the plasma level of transgenic mice over-expressing heparanase. The increased levels of TFPI noted in some cancer patients might reflect the overexpression of heparinase. (307) So heparinase contributes to activation of blood coagulation by inducing TF expression, increasing TF activity and releasing TFPI from endothelial cell surface.

Inflammation induces coagulation:

In a study by von Bruhl et al (154) it was suggested that massive leukocyte accumulation precedes the development of DVT in response to perturbed venous blood flow. In a subanalysis of the Vienna CATS study dataset it was shown that an increase of $1x10^9$/L in

WBC was associated with a 7% increase in VTE risk. (270) A key effect of inflammatory mediators is to induce TF expression on the surface of circulating monocytes, macrophages and neutrophils increasing the pool of potentially available TF. On exposure to inflammatory cytokines, leukocytes are also more prone to microvesiculation and hence capable of producing TF-MPs which may be recruited to growing thrombi via PSGL1- P-selectin interactions and may stabilize the thrombus. Neutrophils can also recruit TF-MPs while the NETS they expel has been shown to serve as an adherence site for tumor derived TF-MPs. (308) Neutrophils in particular are found in large quantities in the plasma of cancer patients and are recruited in the earliest stage of an inflammatory response. Tumor-induced neutrophils have been shown to be more prone to NET formation than their normal counterparts both in leukemia and solid tumors in experimental models. (289)
NETs have bene identified in a number of cancers including pancreatic cancer. Their role in CAT has recently been challenged by Nouboussie et al who argue that it is the constituent products of NETs (cell-free DNA and histones) and not intact NETs that are able to activate coagulation. This observations have only been made in the invitro setting. (309) Neutrophils do not represent however the only source of cfDNA in plasma and other forms may cfDNA may contribute towards a prothrombotic state. Elevated levels of cfDNA in cancer have been characterized as tumor derived or released from injured host blood or vascular tissues via apoptotic and necrotic cell death and are thought to provide an activation surface for the contact pathway of coagulation.

Date et al in propose a putative mechanism of the development of VTE in cancer which may however be of more relevance to pancreatic cancer. In the context of an already hypercoagulable state, inflammation may provide the trigger to drive the procoagulant response. TF levels remain the key factor and the additional effect of inflammation invokes optimal thrombus growth which overcomes the physiological threshold set by the in vivo anticoagulant mechanisms. (308) Of course clinical factors associated with malignancy also increase VTE risk such as vascular stasis due to immobilization or obstruction of blood flow by the tumor, sepsis, DIC< advanced age, thrombocytosis or leukocytosis, certain antineoplastic agents, erythropoiesis stimulating agents and central venous catheter insertion.

5.0 Thromboprophylaxis and Cancer

5.1 Treatment of cancer associated thrombosis:

LMWHs have long been the first choice for long-term treatment of cancer associated thrombosis. Direct oral anticoagulants have more recently been introduced in clinical practice and are an attractive alternative. Large non-inferiority trials have led to their approval for the use in the treatment of VTE and post hoc analyses have suggested that DOACs were either of comparable efficacy and safety or that they were associated with slightly higher bleeding rates. (310) (311)

Recently updated American Society of Clinical Oncology (ASCO) 2019 guidelines recommend the use of LMWH, UFH, fondaparinux or rivaroxaban in patients who do not have severe renal impairment. For long term anticoagulation LMWH, edoxaban or rivaroxaban for at least 6 months are preferred over vitamin K antagonists because of improved efficacy. Caution is recommended with the use of DOACs due to the highest risk of major bleeding events with Gastrointestinal and Genitourinary cancers and the potential drug-drug interactions. Anticoagulation with LMWH, DOACs or VKAs beyond the initial 6 months should be offered to select patients with active cancer (like those with metastatic disease or those receiving chemotherapy). (271) DOACs that target thrombin (direct thrombin inhibitor, dabigatran) or activated factor X (antifactory Xa inhibitors, rivaroxaban, apixaban and edoxaban) are now approved for the treatment of DVT or PE as well as for DVT prophylaxis following orthopedic surgery and for reducing the risk of stroke and systematic embolism in patients with non-valvular atrial fibrillation. Edoxaban and rivaroxaban have been added as VTE treatment options based on evidence from two RCTs. (312, 313) Apixaban and dabigatran do not have published data in comparison with LMWH in the therapeutic setting and are not recommended in the cancer setting until efficacy and safety data are available. For the cases of recurrent VTE while on anticoagulation the expert panel recommends initially checking treatment compliance, heparin induced thrombocytopenia or any evidence of mechanical compression resulting from malignancy.

Management options then include treatment with alternative anticoagulant regimen or increasing the dose of LMWH. The addition of a vena cava filter to LMWH should be reserved as a last result. Higher doses of LMWH can be tried if standard doses have failed but evidence to support these strategies are limited. Populations that require special consideration are patients with renal impairment who are at high risk of bleeding particularly those with concurrent cancer. Studies indicate that enoxaparin requires dose reduction but tinzaparin may not. (314-316) The safety and dosing data for DOACs regarding renal and liver dysfunction have not been studied in detail and are evolving with extensive real-world use. The ISTH SCC recently published a consensus document which also supports that DOACS should be the first option for cancer patients with acute VTE provided they have a low risk of bleeding and no drug-drug interactions with concomitant treatment and LMWH should be used for cancer patients with acute VTE and high risk of bleeding. (317)This consensus is in agreement with the ASCO 2019 guidelines. The use of DOACs has also entered as an alternative treatment in the recent NCCN guidelines. Panel members categorized as level 1 the use of deltaparin and LMWH followed by edoxaban for the treatment of CTE. The use of other DOACs has been categorized as 2A. NCCN guidelines list urinary or GI tract lesions, pathology or instrumentation as relative contraindications to DOACs in patients with cancer. (318)

5.2 Thromboprophylaxis for Cancer associated thrombosis

5.2.1 Clinical trials and parenteral anticoagulation:

A Cochrane 2017 review summarized the absolute risk reduction from all the trials combined that involve parenteral anticoagulation in ambulatory patients with cancer; a reduction in symptomatic VTE (relative risk [RR], 0.56; 95% CI 0.47-0.68) and an increase in major bleeding (RR 1.30; 95% CI 0.94-1.79) that did not reach statistical significance.

The following trials have compared LMWH with no thromboprophylaxis (observation or placebo) in ambulatory cancer patients.

- PROTECHT: The Prophylaxis of Thromboembolism during Chemotherapy trial assigned 1150 patients to LMWH nadroparin (3800 anti-Xa international units

subcutaneously once daily) versus placebo for VTE prevention for the duration of chemotherapy and up to a maximum of 4 months (patients with lung, breast, gastrointestinal, ovarian or head and neck cancer and PS ≤ 2). Compared with placebo patients on nadroparin had a lower incidence of thromboembolic events (venous and arterial) (3.9% with placebo versus 2% with nadroparin). The risk of bleeding was similar between the two groups. (267)

- In the CONKO-004 study in patients with pancreatic cancer, VTE rates were 5% in the LMWH enoxaparin arm (1 mg/kg per day for 3 months, then 40 mg per day) compared with 14.5% in the observation arm (*P* <0.01). (319)
- The FRAGEM study compared LMWH deltaparin ((therapeutic weight adjusted dose for 12 weeks) to observation in pancreatic cancer patients also. VTE rates were 12% in the LMWH deltaparin arm and 31% in the chemotherapy-alone arm (*P* = 0.019). (320)
- SAVE-ONCO: This trial randomly assigned 3212 patients with metastatic or locally advanced cancer at chemotherapy initiation to ultra-LMW heparin semuloparin (20mg once daily) versus placebo. The risk of VTE compared with placebo of those receiving semuloparin was lower (3.4 with placebo versus 1.2 percent with semuloparin; HR 0.36; 95% CI 0.21-0.60. The incidence of major bleeding was similar.

No cancer-specific randomized controlled trials for thromboprophylaxis have been carried out in hospitalized patients but use of thromboprophylaxis has been shown to decrease DVT with no increase in major bleeding in three trials (MEDENOX, PREVENT, LIFENOX) of acutely ill medical patients (5-15% of the cohort were cancer patients). Despite lack of cancer-specific data current guidelines recommend thromboprophylaxis for hospitalized patients who are high risk based on extrapolation data from the three positive studies. (321-323)

5.2.2 DOACS and data on thromboprophylaxis for cancer patients:

There are data from clinical trials on the use of anticoagulants in the prevention of VTE in ambulatory cancer patients that compare LMWH to placebo or DOACs to placebo. Data

from clinical trials that compare LMWH to DOACs or different factor Xa inhibitors with each other are only just now becoming available. (table 16). Most trials have shown a reduction in symptomatic VTE with anticoagulation and the risk reduction is greatest for those with the highest baseline VTE risk.

Two trials published in 2019 addressed the safety and effectiveness of a direct factor Xa inhibitor in patients with Khorana score ≥2 receiving chemotherapy. Placebo-controlled, double blind trial (AVERT) compared 2.5mg apixaban twice daily for thromboprophylaxis in ambulatory cancer patients with cancer who were at intermediate-to-high risk for venous thromboembolism (Khorana >=2) and were starting chemotherapy and demonstrated a significantly lower rate of VTE in the apixaban versus the placebo group (273). It randomly assigned 574 ambulatory patients with Khonara score ≥2 and were starting chemotherapy to receive apixaban at the prophylactic dose versus placebo for 180 days. Apixaban resulted in a 6% absolute risk reduction in VTE (from 10.2 % with placebo to 4.2 percent with apixaban in a modified intention-to-treat analysis [number needed to treat 17]; hazard ratio [HR] 0.41; 95% CI 0.26-0.65; adjusted odds ratio [OR] 0.39; 95% CI 0.20-0.76). Apixaban was associated with an increase in major bleeding (3.5 % versus 1.8% with placebo, number needed to harm 59, HR 2.0; 95% CI 1.01-3.95). The major bleed rate was not significantly higher during the treatment period and mortality was 12% with apixaban and 10% with placebo most deaths being associated with cancer progression. (273) This trial focused only on symptomatic VTE or incidental PE and did not screen at baseline for DVT.

The CASSINI study (randomized, double blind, control trial) randomly assigned 841 individuals with caner who had a Khorana score ≥2 and were starting chemotherapy to receive rivaroxaban (10mg once daily) versus placebo for 180 days. Rivaroxaban versus placebo resulted in 2.8% absolute risk reduction in VTE (from 8.8% with placebo to 6% with rivaroxaban; HR 0.66; 95% CI 0.40 -1.09) in the intention to treat analysis. Many of the thrombotic events occurred when patients were off anticoagulation. Rivaroxaban was associated with increased risk of major bleed that did not reach statistical significance and a reduction in mortality that barely reached significance (ClinicalTrials.org Identifier: NCT02555878) (324). In the CASSINI trial patients were screened at baseline and every 8 weeks during the study using bilateral leg ultrasound so 4.5% of patients were nor

randomized after detection of subclinical proximal DVT. CASSINI trial also did not require systemic chemotherapy and other forms of systematic cancer therapy was allowed.

Apixaban is also being compared (at 2.5mg twice daily for 6 months) to placebo for primary prevention of VTE in patients with multiple myeloma receiving immunomodulatory agents without a history of prior VTE and positive data have become available. At 3 month interim analysis a pilot phase IV study on 50 patients with MM on IMiDs and apixaban showed that no patients experienced VTE, major hemorrhage, stroke or MI. (325) A combined analysis of the data from the two trials found a small absolute reduction in the risk of VTE (2.5 percentage points, number needed to treat 24) with a small increase in the risk of major bleeding (number needed to harm 77). These data contributed to the decision to restrict anticoagulation for primary VTE prophylaxis mainly to individuals at especially high VTE risk (these with a Khorana score 3 or higher) as they are likely to have the greatest absolute risk reduction. (326) The concerns associated with these two trials are that the Khorana score does not take into consideration the specific chemotherapy regimen and most types of common cancer (colorectal, breast and prostate) are underrepresented in these trials. One must therefore be careful prior to extrapolation of these data to the general population.

Study identifier	Population	Intervention	Comparator	Primary clinical outcome	Follow-up	Sample size	Study	Estimated completion date
EINSTEIN CHOICE (NCT02064439)	secondary VTE prevention	rivaroxaban 10mg or 20 mg OD	aspirin 100 mg OD	recurrent VTE, major bleeding	12 months	3399	phase 3b	Oct-16
HOME-PE (NCT02811237)	outpatient management of acute PE	management based on the Hestia rule	management based on the sPESI	recurrent VTE, major bleeding, death	30 days	1975	phase 3b	Jun-18
HOT-PE (2013-001657-28)	outpatient management of acute PE	rivaroxaban standard therapeutic dose		recurrent VTE or PE related death	3 months	1100	phase 4	Jan-18
RIDTS (NCT02722	isolated distal DVT	Rivaroxaban standard therapeutic dose for 6 weeks after an initial course of 6 week treatment	placebo	recurrent VTE	3 months	1100	phase 3b	Jun-20
Hokusai VTE cancer	cancer patients with acute VTE	edoxaban standard dose	deltaparin standard therapeutic dose	recurrent VTE, clinically relevant bleeding	6 months	1000	phase 3b	Dec- 1017
VERDICT (NCT02664155)	acute VTE in patients with moderate or several renal dysfunction	apixaban standard therapeutic dose for 7 days followed by 2.5 mg daily or rivaroban standard therapeutic dose 21 days followed by 15 mg once daily	standard of care	net clinical benefit	3 months	800	phase 3b	Mar-19
PEITHO-2 (NCT02596555)	intermediate risk PE	LMWH standard therapeutic dose for 72 hours followed by dabigatran standard therapeutic dose		recurrent VTE or PE related death	6 months	700	phase 4	Aug-19
TRAPS (NCT02157272)	antiphospholipid syndrome	rivaroxaban standard dose	INR-adjusted warfarin	recurrent thrombosis, major bleed, death	4 years	536	phase 3b	Dec-18
SELECT-D (2012-005589-37) (313)	cancer patients with acute VTE	rivaroxaban standard therapeutic dose for 6 months rivaroxaban standard therapeutic dose for further 6months	deltaparin 200 IU/kg daily for 1 month followed by 150 IU/kg during months 2-6 placebo	recurrent VTE or PE related death	6+6 months	530	phase 3b	Dec-18
Apixaban: VTE treatment in cancer (NCT02585713)	cancer patients with acute VTE	apixaban standard therapeutic dose	deltaparin standard therapeutic dose		6 months	315	phase 3b	Dec-20
RAMBLE (NCT02761044)	treatment of VTE in young women	rivaroxaban standard therapeutic dose	apixaban standard therapeutic dose	patient reported menstrual bleeding	3 months	308	phase 3b	May-19
CAP (NCT02581176)	cancer patients with acute VTE	apixaban standard therapeutic dose for 6 months followed by 2.5 mg daily		recurrent VTE major, clinical relevant non	6 months	300	phase 4	Jan-21

				major bleeding				
CASTA-DIVA (NCT02746185)	cancer patients with acute VTE	rivaroxaban standard therapeutic dose	deltaparin standard therapeutic dose	recurrent VTE	6 months	200	phase 3b	May-17

Table 16: ongoing clinical trials for the use of DOACS/NOACs for the prevention of cancer associated thrombosis.

5.2.3 Guidelines for the prevention of CAT:

Guidelines for the prevention of CAT in medical patients have been published by the American Society of Clinical Oncology (ASCO), the European Society of Medical Oncology (ESMO), the National Comprehensive Cancer Network (NCCN) and the International Clinical practice Guidelines and recommend the use of parenteral anticoagulants for pharmacological thromboprophylaxis. Recently NOACs have been included in the most updated version of ASCO guidelines. (Table 17) The Scientific and Standardisation Committee of the International Society on Thrombosis and Haemostasis (ISTH) does not routinely recommend thromboprophylaxis ambulatory cancer patients undergoing chemotherapy when they are deemed to be low risk for VTE. Current consensus guidelines discourage the use of individual risk factors in clinical decision making. Use of the Khorana score is recommended for the identification of patients at high risk for VTE.

Recent guidelines were published by the American Society of Clinical Oncology in August 2019 regarding thromboprophylaxis in cancer patients. (271) Guidelines revise previously published recommendations and the most notable change is the addition of direct oral anticoagulants (DOACs) or non-vitamin K antagonist as options for VTE prophylaxis and treatment. A total of 26 meta-analyses and nine RCTs (273) (312) (327) (328) (313) (274) were reviewed and 18 publications regarding VTE risk assessment models were identified.

Class	Prophylaxis
ESMO (329)	• Surgery: LMWH, UFH, or fondaparinux • Inpatients: UFH, LMWH, or fondaparinux in hospitalised patients confined to bed • Chemotherapy: LMWH or warfarin in myeloma patients receiving thalidomide plus dexamethasone or thalidomide plus chemotherapy
NCCN (330)	• Surgery: LMWH, fondaparinux, UFH, or warfarin • Inpatient: LMWH, fondaparinux, UFH, or warfarin • Low-risk myeloma patients (outpatient): Aspirin • Chemotherapy: LMWH or warfarin in myeloma patients receiving lenalidomide or thalidomide + dexamethasone and chemotherapy
ISTH (331)	• Low-risk outpatients: Routine prophylaxis is not recommended • High-risk outpatients: LMWH for patients with solid tumours (except brain tumours) and a Khorana Score of >= 3 • Advanced pancreatic cancer: LMWH for patients with advanced pancreatic cancer starting or receiving systemic therapy • Chemotherapy: LMWH or aspirin in patients with myeloma receiving thalidomide-based or lenalidomide based combination regimens
ASCO (271)	• Inpatient: Pharmacologic thromboprophylaxis in the absence of bleeding or other contraindication is recommended (UFH, LMWH) • Perioperative: ULF or LMWH unless contraindicated should be offered • Chemotherapy: apixaban, rivaroxaban or LMWH should be offered for patients with a Khorana score >= 2 provided there are no significant risk factors for bleeding and not drug interactions.

Table 17: Current guidelines for the prevention of Cancer associated thrombosis. ESMO: European society of medical oncology; NCCN: National Comprehensive Cancer network; ISTH: International Society of Thrombosis and Hemostasis; ASCO: American Society of Clinical Oncology; LMWH: low molecular weight heparin; UFH: Unfractionated heparin.

Regarding thromboprophylaxis in hospitalized patients with active malignancy the published inpatient trials have enrolled mixed populations including patients with cancer and general medical patients and most patients have serious medical conditions that make

it difficult to generalize and extrapolate the data. There are no cancer-only specific trials that evaluate inpatient thromboprophylaxis. Hospitalized patients with cancer often have risk factors in addition to immobility. Choice of thromboprophylaxis based on risk assessment models may enhance its appropriate use. (54,56) *The recommendation is that hospitalized patients with active malignancy without additional risk factors may be offered pharmacologic thromboprophylaxis in the absence of bleeding or other contraindication.*

The recommendation is also that all patients with malignant disease undergoing major surgical intervention should be offered pharmacologic thromboprophylaxis either unfractionated heparin (UFH) or LMWH unless contraindicated. Pharmacologic thromboprophylaxis should be continued for 7-10 days in all patients and should be extended for up to 4 weeks in patients who have high risk features (restricted mobility, obesity, VTE history etc) or undergo major abdominal or pelvic surgery. There is currently insufficient evidence to support VTE prophylaxis in patients admitted for minor procedures or those undergoing stem cell or bone marrow transplant.

Regarding the use of thromboprophylaxis during systemic chemotherapy for ambulatory patients the recommendation is not to offer routine pharmacologic thromboprophylaxis to all outpatients with cancer. Patients with a Khorana score of 2 or higher prior to starting a new systemic chemotherapy regimens may be offered thromboprophylaxis with apixaban, rivaroxaban or LMWH provided there are no significant risk factors for bleeding and not drug interactions. A special mention is made for patients with MM who receive thalidomide or lenalidomide based regimens with chemotherapy and/or dexamethasone who should receive aspirin if considered low risk and LMWH if considered high risk.

In terms of available literature and evidence to support this recommendation there are initial clinical trials which focused on the efficacy and safety of LMWH in unselected cancer patients without risk stratification and more recent trials testing DOACs in high-risk patients. The greatest absolute risk reduction with the use of LMWH has been seen in pancreatic cancer or selected high risk patients (the FRAGEM (320) and CONKO-004 (332) clinical trials

focused specifically on this patient population). In a pooled result analysis from the phase II PHACS study and two of the largest phase III studies, PROTECTH and SAVE-ONCO in selected high risk patients based on the Khorana risk score revealed a reduction in VTE from 8.1% to 3.3% with the use of LMWH. (272)

5.2.4 Decision making for thromboprophylaxis:

Anticoagulation with direct factor Xa inhibitors or LMWH can reduce VTE risk but at the same time increases the risk of bleeding. As a result in practice thromboprophylaxis is mostly individualized. For individuals with relatively low risk of VTE (Khorana score <2) no thromboprophylaxis is recommended for primary VTE prevention. For those with high VTE risk, greater absolute risk reduction is expected and therefore thromboprophylaxis is recommended for those with Khorana score ≥3 or those with a score of 2 who place a higher value on avoiding VTE than bleeding. A number of factors need to be considered in this individualized approached. In terms of bleeding risk it is higher in cancer patients than in other patients. Gastrointestinal, gynecological tumors and brain metastases are associated with particularly high bleeding risk and concomitant medications such as aspirin, NSAIDs or steroids also increase the risk. Additional risk factors such as use of antiplatelet agents, renal or hepatic impairment, thrombocytopenia or prior history of GI bleeding should be taken into consideration. Given the increased risk for major bleeding events associated with DOACs, LMWH are preferred in settings with increased bleeding risk. More data is however required to provide more specific information. Anticoagulation is also associated with practical issues such as extra cost, difficulties with the administration of subcutaneous injections and bleeding complications that may interfere with scheduled chemotherapy administration. Temporary interruption for thrombocytopenia associated with myelosuppressive chemotherapy or other invasive procedures should also be considered. Guidelines on thromboprophylaxis for patients with thrombocytopenia are based on data from small groups of patients. Standard doses of agents are recommended for platelet count ≥ 50 $x10^3$/ L in the absence of haemorrhagic diathesis and no other hemostasis disorders. For platelet counts 25 - 50 $x10^3$/ L decision should be based on

analysis of risk and benefits. Renal function should also be taken into consideration based on each agents summary of product characteristics and individual patient assessment of risks and benefits. Another important safety issue associated with the use of DOAC is drug-drug interactions which should be taken into careful consideration prior to initiation of treatment. It should also be noted that features of the Khorana score might change over time (hemoglobin, white blood cell count and platelet count). Optimal duration of therapy is still unknown and optimal dosing in individuals with very high BMI is also unknown.

Some wonder whether thromboprophylaxis offers survival advantage in patients with cancer through a potential direct anti-tumor effect. Data is overall mixed and there are no clinical trial data to support the use of anticoagulation to prolong survival through a direct anti-tumor effect in the absence of another indication. Several meta-analyses of randomized trials comparing LMWH with placebo have not found an improvement or reduction in survival. (333) (334)For oral anticoagulants (warfarin and direct factor Xa inhibitors) Cochrane reviews and recent RCTs have not found any survival benefit. (273) (335) Despite the presence of guidelines and the availability of effective methods for thromboprophylaxis global audits have shown underuse in hospitalized patients at risk and in cancer patients on chemotherapy.

6.0 Venous thromboembolism and Multiple Myeloma

6.1 Epidemiology

Venous thromboembolism (VTE) remains one of the common complications in patients with multiple myeloma (MM). (336) Approximately 10% of patients with newly diagnosed MM (NDMM) will develop VTE during their disease course. (337-339) and the incidence is reported to range between 8-22 per 1000 person-years. According to an older report on the incidence of thrombosis in patients with hematological malignancies, the crude rate of DVT during an 8 year follow up of more than 6000 MM patients was 8%. (340)

The most common venous thrombosis observed in MM is deep vein thrombosis (DVT) in the lower extremities, followed by central venous catheter related thrombosis, pulmonary embolism and arm vein thrombosis. . (341) (342) The risk is higher during the first months of therapy (time-dependent) and in newly diagnosed MM (NDMM) as compared to relapsed and recurrent MM (RRMM). This early high-risk effect can be explained by the thrombogenic effect of the treatment agents or more active disease and higher disease burden which can be linked to stronger effect of the procoagulant intrinsic mechanisms. (338) A Swedish study compared 18627 MM patients to 70991 controls and found the risk of VTE to be 7.5 times higher after 1 year of follow up, 4.6 times after 5 years and 4.1 after 10 years. (339) A retrospective US based study assessed the one-year incidence of VTE in patients with MM and the time-varying treatment exposure effects. The overall incidence of VTE was 107.2 per 1000 person-years and one-half of the events occurred during the first 90 days. The study found few baseline factors (age, male gender and some comorbidities) to be associated with VTE risk pointing to a more significant impact from treatment related effects (IMiDs, CVC, EPO, infection and hospitalizations) . (343)

There is no data on the incidence of VTE in patients with smoldering MM but there are a few reports on rates of VTE and the pre-malignant condition of monoclonal gammopathy of undetermined significance (MGUS). Data is conflicting with regards to the risk of VTE

associated with this pre-malignant state but most reports point to an increased risk of VTE in patients with MGUS. Three population based registry studies report higher VTE rates compared to the general population. In a US veteran man-only registry the relative risk of VTE in patients with MGUS was found to be 3.3 times higher relative to the population. (341) A Danish registry compared patients with MGUS to matched individuals in the general population and reported hazard ratios of 3.4, 2.1 and 2.1 for venous thrombosis at 1 ,5, 10 years after MGUS diagnosis and the risk did not vary by M-protein concentration. (340) In the Swedish study the rate was lower, 1.4 times higher. (344) In a prospective study of 310 MGUS patients the VTE rate was 6.1% (345) and in a retrospective follow-up of 174 MGUS patients over a 10-year period it was 7.5% (346). Some of the above studies have identified significant associations between the level of M-protein, age and progression to malignancy. (345)

MGUS rates are however higher in individuals tested for other medical conditions and therefore the above studies might not be truly population-based studies and VTE rates reported not be very accurate. Another population-based study by Bida et al in 2009 did not confirm an association (347). In a more recent multicenter cohort study of 1491 the incidence of VTE was 1.9 per 1000 person years (2.7% during the follow up period). Multivariate analysis showed an increased VTE risk associated with serum monoclonal (M) -protein levels >1.6g/L at diagnosis. (348) No thrombosis was recorded in those patients who developed eventually SMM or MM. Overall the incidence of venous thrombosis in patients with MGUS was not higher compared to that reported in the general population.

Contrary to the clear adverse impact on overall survival associated with VTE in solid tumors, many studies don't show inferior survival with VTE in patients with MM. (324) Other however do link VTE with inferior overall survival in MM patients (349) (350) (338). This finding might point towards different underlying prothrombotic mechanisms in MM compared to solid tumors. (324) (349, 351) Kristinsson et al in 2012 reported an inferior survival in a population-based Swedish study with a higher mortality at 1-5, 5- and 10 years follow up compared with those without VTE although arterial thrombosis is more clearly associated with a higher risk of death. (349) Thrombotic events do however have an adverse

impact as they lead to treatment interruption, increased morbidity and add to the economic burden of the disease in the population. (352, 353) The underlying pathogenesis of increased VTE risk is not fully understood yet and despite appropriate risk assessment and thromboprophylaxis the rates remain high (15-24% in the highest risk group). (354-356)

There is a lack of studies that specifically assess the economic burden of VTE occurrence in patients with MM. Data from other cancer patients demonstrated increased costs associated with the long term use of pharmaceutical agents for treatment of thrombosis, the need of hospitalization and increased risk of complications as well as adverse effects on patient's quality of life. Khorana et al published a report on the health care costs associated with VTE in high-risk ambulatory patients with bladder, colorectal, lung, ovarian, pancreatic, or gastric cancer, who receive chemotherapy in the US. Patients with VTE were identified and matched to control (non VTE) cancer patients. Patients with VTE had three times as many all-cause hospitalizations compared to non-VTE patients. Outpatient medical claims and prescription claims were also higher. Total health care costs were mean USD 74,959 versus USD 41,691 per patient; *P* , 0.0001 over the 12 month post VTE period. (352)

Given the unprecedented improvements in the OS of patients with MM mostly due to the availability of new drugs over the last decade the conversation regarding the price and affordability of treatment is becoming increasingly relevant. Formal pharmacoeconomic analyses are required to assess cost-effectiveness of treatment options and the financial burden of managing the complications and adverse effects of these therapeutic agents, including the management of VTE. (357)

The necessity of effectively addressing thrombotic complications in MM patients has become more evident with the use of immunomodulatory drugs (IMiDs) in the treatment of MM. Conventional therapies such as melphalan and prednisone are associated with a 1-2% risk of VTE. IMiD monotherapy increases this risk further to approximately 3-4% at diagnosis and 1-2% at relapse. However, the combination of IMiDs with high doses of dexamethasone or chemotherapeutic agents has a multiplicative effect that increases VTE rates up to 26%. (338) (339) (358) (359).

6.2 Risk factors for Venous thromboembolism in Multiple myeloma

The thrombogenicity in myeloma is multifactorial and risk factors associated with VTE occurrence in MM patients have long been distinguished in three groups. (360, 361) Patient-related clinical risk factors, myeloma-related risk factors and finally risk linked to the type of anti-myeloma treatment administered. The treatment regimen of choice is one of the major determinants of VTE risk; the standard 1-2% VTE rates associated with conventional regimens such as melphalan and prednisone increase to about 4% with immunomodulatory drug monotherapy and up to 26% in some reports when immunomodulatory agents (IMiDs) are combined with high dose corticosteroids or multi-agent chemotherapy (338, 358).

Patient related risk factors			
Age	Brown et al 2016 (362) hazard of thrombosis for the 35–64 and 65–74 age groups compared to the 18–34 reference group, HR 2.8 for the 75+age group (1.6-4.8 95% CI)	Baker et al 2018 (363) Age not identified as risk factor for VTE (p=0.56)	Bagratuni et al 2013 (364) n=200, VTEs were more frequent in patients >65 years (8.1% vs. 1.6%)
Body mass index ≥30 kg/m^2 Family history Race	No specific studies in MM for these risk factors		
Personal history	Anaissie et al 2012 (365) history of VTE was a strong predictor of VTE on univariate analysis (P < .000005) n=604		
Cardiac disease (eg, symptomatic coronary artery disease, congestive heart failure, or history of stent placement/CABG)	Brown et al (362) congestive cardiac failure associated with hazard HR = 1.7 (95% CI, 1.4–2.1), Hypertension associated with hazard (HR = 1.2 (95% CI, 1.0–1.3)		

Other Comorbidity: Diabetes mellitus, renal impairment, liver impairment, chronic inflammatory disease, COPD, immobilization, autoimmune disease Recent trauma or surgery Hospitalization Immobility Inherited thrombophilia Use of hormone replacement Acute infection			No specific data on these risk factors in patients with MM available			
Use of erythropoietin	Chalayer et al 2018 (366) OR 0.49 (95% CI 0.18-3.83)	Anaissie et al 2012 (365) n=604 prophylactic EPO (P = 0.002; OR, 2.488; 95% CI, 1.432-4.324)	Menon et al 2008; (367) n=125, no difference in VTE rate with EPO use (4.8% and 8.6%, respectively (*P* = .54)	Knight et al 2015; (171) n plus lenalidomide: OR 3.21 (1.72-6.01 95% CI, p<0.001)	Galli et al 2004; (368) n=199, 8.1% prevalence with EPO vs 9.3% without, p>0.5)	Leleu et al 2013 (324) Relative RIsk of VTE 3.46 (0.45-3.7 95% CI, p=0.04)
Central venous catheter or pacemaker	Cortelezzi et al 2005 (369) 12% VTE events in 416 patients with hematologic malignancies and CVC insertion (MM diagnosis seen in 18.8% of pts)					
Disease specific risk factors						
New diagnosis of MM	Zangari et al 2003; (370) (n=535) newly diagnosed disease (OR, 2.5; P = 0.001)					
Chromosome 11 abnormalities	Zangari et al 2003; (370)(n=535) (OR, 1.8; P = 0.048)					
	No data on these biomarkers and VTE risk					
Microparticle (MP) associated Tissue factor and Tissue factor (TFP	Auwerda et al 2011 (252): (n=122) NDMM; MP-TF levels prior to treatment initiation did not predict VTE but MP-TF remained elevated in patients who developed VTE 15.1 [10.3-25.2], in contrast to patients not developing VTE (11.4 [7.0-25.2], P<0.001				Elabedin et al 2012 TF levels >53.3 (AUC 0.88 95% CI 0.8-0.9) associated with DVT occurrence (p=0.0001)	
Thrombin lag phase (s)	Undas et al 2015; (371) 60 [52–60.5] vs 50 [47–55] , p=0.01 In patients with VTE					

Thrombin peak concentration (nmol/l)	Undas et al 2015; (371) higher Peak concentration associated with VTE; 503.5 [418–550] vs 344.8 [269–411] in patients without VTE, p <0.001	Leiba et al 2017 (372) higher peak height values (620 vs 400 nM, p<0.001) associated with higher VTE risk	Chalayer et al 2018 (366) 186 nmol/L for patient with VTE vs 149nmol/L for not VTE, p=0.22 in univariate analysis	Ay et al 2011 (238) associated with VTE risk	
Time to peak at baseline (min)	Chalayer et al 2018 (366) at baseline; 10.8min for patients with VTE vs 9min for no VTE, p=0.82 in univariate analysis, no significant association with VTE			Ay et al 2011 (238) associated with VTE risk	
Endogenous thrombin potential (ETP) (Mxmin)	Dargaud et al 2019; ETP higher in MM patients versus controls (373)		Ay et al 2011 (238) not associated with VTE risk	Leiba et al 2017 (372) higher EPT (2896 vs 2028 nMxmin, p<0.001) associated with higher VTE risk	Chalayer et al 2018 (366) increase in ETP between baseline and cycle 4 – no association with VTE
Thrombin-activatable fibrinolysis inhibitor (TAFI) (mg/ml)	Undas et al 2015 (371)Higher levels associated with VTE 45.3 [44.6–47.4] vs 38.9 [33.5–42.3] <0.001				
Plasminogen activator inhibitory PAI-1 act (IU/ml)	Undas et al 2015; (371)higher PAI-1 levels associated with VTE Risk 11 [9.9–12.8] vs 8.3 [6.4–10.5], p=0.004				
Lower clot permeability and clot lysis	Undas et al 2015; (371)in patients with lower clot permeability Ks (10^{-9} cm^2) and lower D-D_{rate}, (maximum rate of increase in D-dimer levels in the lysis assay) associated with higher VTE risk				

Acquired Activated protein C resistance (APC-Res)	Zangari et al 2002; (374) higher proportion of patients with APC resistance developed DVT (5/14 versus 7/38; P = 0.04) - 41.7% prevalence of APC-R in the group of NDMM who developed VTE	Cini et al 2010; (375) no difference in VTE occurrence between patients with APCR (6.7% vs 10.3%, p=1.0)	Elice et al 2006 (376) higher incidence of VTE with aAPCR ; 1178 patients; 31% versus 12%; p <0.001)
NFκB1 gene Single nucleotide polymorphism	Bagratuni et al 2013 (364) NFκB1 and VTE risk: OR 3.76, 95%CI 1–16, P=0.051		
Factor V Leiden (R506Q) or G20210A prothrombin mutation	Cini et al (375) patients with polymorphisms had not increased VTE rate (10% vs 9.4%, p=0.27)	Bagratuni et al 2013 (364) FVLeiden and FIIG20210A not associated with higher VTE rates	
P-selectin (ng/ml)	Ay et al 2008 (246) Elevated P-selectin (>53.1ng/ml) risk factor for VTE (HR= 2.6, 95% CI, 1.4-4.9, P = .003)		
vonWIllenbrand (WVF) increased levels	Minnema et al 2003 (377) N=19 patients on thalidomide VWF-Ag in patients with VTE was 375 ± 121% vs. 235 ± 116% in patients without VTE (*P* = 0.03)	Van Marion et al 2008 (378) higher levels of VWF not associated with VTE OR 2.69 95% CI 0.71-10.26, p=0.147	
FVIII (factor VIII)	Minnema et al 2003 (377) N=19 patients on thalidomide FVIII:C was 352 ± 67% vs. 283 ± 114% in patients without VTE (*P* = 0.17)	Cini et al 2010: (375) elevated FVIII activity not associated with higher VTE rate (10% vs 7.4% p=0.76)	Van Marion et al 2008(378) higher levels of FVIII not associated with VTE occurrence
Myeloma Therapy Related			
IMiD in combination with (2 points) · High-dose dexamethasone (>480mg/month) · Multi-agent chemotherapy · Doxorubicin IMiD alone (1 point)			

Table 18: Risk factors associated with Venous thromboembolism in Multiple myeloma and studies that have reported the relevant association. IMiD: Immunomodulatory agent

Table 18 summarizes the main risk factors associated with VTE in patients with MM; they are categorized as patient related, disease related and treatment related

6.2.1 Patient Related Risk factors:

Common risk factors of VTE apply, like advanced age, BMI >30, personal or family history of VTE, presence of central venous catheter, thrombophilia, co-morbidities such as cardiac disease, chronic obstructive pulmonary disease, chronic inflammatory diseases, autoimmune disease, chronic renal or liver disease, also trauma and recent surgery (<6 weeks), immobility (and increased venous stasis), acute infections and use of tamoxifen or hormone replacement. One study assessed multiple VTE risk factors in patients with hematologic malignancies and report 12% VTE events in 416 patients with hematologic malignancies and CVC insertion (MM diagnosis in the overall population was in 18.8% of patients). (369)Median age of diagnosis of MM is 67-70 years old. Renal impairment and immobility due to bone disease (or acute infections and hospitalization further increase the VTE risk in myeloma patients. In a study of 190 patients younger than 65 years old with NDMM classic genetic thrombophilic polymorphisms (Factor V Leiden and PTG20210A polymorphism) were superimposable to that of the normal controls. Genetic thrombophilic abnormalities were found in 5.3% (3.2% FVL and 2.1% PTG20210A) of patients and were associated with a two-fold increase in relative risk of VTE but there was no difference in comparison with the population. (375) FVLeiden and FIIG20210A were not associated with higher VTE rates in another study by Bagratuni et al. (364) These are all well recognized VTE risk factors and there are no studies specific to MM that demonstrate their association with increased risk of venous thromboembolism.

6.2.2 Disease Related Risk factors, Pathogenesis and potential biomarkers linked to VTE:

Up to date the pathogenesis of thrombosis in MM has not been delineated. A number of groups have studied mediators of cellular and plasma coagulation in MM patients, have compared them to healthy controls, prior to and during treatment and have studied their association to VTE occurrence.

None has been identified yet as a biomarker that can be incorporated in risk assessment and therefore none is included in the IWMG or EMN risk stratification algorithm. The ultimate goal is however to identify a marker of coagulation that reflects increased risk of thrombosis in MM patients sensitively enough to be incorporated in validated risk assessment tools in combination with clinical factors to improve risk stratification and optimize thromboprophylaxis. Ideally a global marker of hemostasis that can be assessed using point of care tests to pinpoint patients with prothrombotic hemostatic profiles or even highlight those with features of resistance to heparin or other anticoagulation needs to be recognized.

The new diagnosis of myeloma as compared to the disease in the relapse setting is a risk factor for venous thromboembolism as it has been demonstrated by data from clinical trials. One study also found an association between light-chain myeloma and presence of chromosome 11 abnormalities with VTE risk. (370)

Very high levels of monoclonal immunoglobulins lead to increased viscosity that in turn disrupts normal coagulation. Reports have linked hyperviscosity with increased risk of venous thrombosis although the extent of this contribution is not clear.(361, 379) MM patients tend to form denser fibrin clots that display poor lysability, permeability and turbidity. It is unclear whether these changed properties are linked to the monoclonal component but there are reports that it interferes with fibrin polymerization and impairs fibrinolysis. (380) (381) (338, 361, 379) Carr et al have previously demonstrated that higher IgG levels induced thin fiber formation (380) whereas Undas et al showed that fibrin clots in MM patients are denser compared to healthy controls (371). Higher M protein concentrations have been also linked to longer prothrombin time (PT). (360, 380) In a study of 48 MM patients, thrombin generation, factor VIII and fibrinolytic proteins were measured prior to and following three months of induction therapy mostly with cyclophosphamide, thalidomide and dexamethasone. After three months they showed improved clot properties, permeability and compaction, shorter lag phase and clot lysis time. Therapy also lowered thrombin generation, antiplasmin and thrombin activated fibrinolysis inhibitor

(TAFI). (371) Increased levels of PAI-1 were confirmed in a study by Bagratuni et al in 2013. (364)

Platelet dysfunction and increased adhesion has been reported in patients with MM which may also explain the demonstrated efficacy of aspirin as an agent for thromboprophylaxis in MM patients. (382) (381) In an early study by Baz et al platelet aggregation was increased in patients receiving thalidomide who developed VTE. (383) One study demonstrated in vitro longer ADP closure time (CT) and Col/Epi CT (indication of platelet dysfunction) which positively correlate with monoclonal IgG protein. In 31 MM patients closure time (CT) was assessed at diagnosis and following 2 and 4 months of treatment with thalidomide (T) or thalidomide plus dexamethasone . Longer CT was described in 30-33% of patients, which significantly shortened after therapy with T or TD. (384) Some reports have also shown Lupus Antibody Coagulant (LAC) like activity by the monoclonal component and the presence of antibodies against other phospholipids, antithrombin, protein C and protein S exist in MM patients. (385, 386) It is postulated that Ig fractions associated with anti-phospholipid antibodies or LAC exacerbate resistance of the activated protein C pathway and ultimately increase the risk of thrombosis. (371, 379, 387)

A hypercoagulable environment in MM is also sustained by increased levels of inflammatory cytokines such as IL-6, TNF-a, VEFG, NF□B. There is upregulation of Tissue factor (TF), fibrinogen, factor VIII (FVIII) and von Willenbrand Factor (vWF) and downregulation of thrombomodulin, endothelial protein C receptor (EPCR), APC (activated protein C), and tissue plasminogen activator (t-PA). (388) High levels of vWF and FVIII have been confirmed by multiple studies; both coagulation factors are strongly elevated and linked to advanced disease stage or active disease. Robak et al found >150% FVIII increase in 78% of MM patients and >160% increase in vWF in 66% compared to controls. In the same study fibrinogen levels were elevated in 50% of MM patients at diagnosis and correlated positively with FVIII and vWF levels. (384) In another cohort of 135 MM patients both vWF and FVIII were increased at diagnosis and remained high after treatment initiation during prospective follow up evaluation. The increase was independent of the treatment regimen received. In the same group of patients fibrinogen was increased at diagnosis and at follow-

up particularly in patients who received thalidomide. (378, 389) Minnema et al also found high VWF-Ag and FVIII levels in patients who received thalidomide and had a VTE compared to those who did not. (377) Another group performed coagulation assessment and found FVIII levels and PC levels were higher in MM (n=24) and MGUS (n=19) patients compared to healthy controls (n=48), VWF were significantly higher in MM group vs MGUs and healthy control group and PS and AT levels were similar across all groups. (390)

Tissue factor derived microparticles (MP-TF) also seem to play a potential role in the pathogenesis of VTE in MM. In one study of NDMM patients MP-TF activity levels were higher at diagnosis of MM compared to controls, decreased after induction chemotherapy and remained elevated in patients who experienced VTE. (252) Interestingly Cesarman analyzed 55 human MM cell lines none of which expressed F3 (TF gene) and TF expression was also absent in actual tumors samples from MM patients. (391)

Recently a Japanese group studied thrombosis related biomarkers in 103 MM patients versus 30 controls and prior and post therapy. Platelet derived microparticles (PDMP), plasminogen activator inhibitor-1 (PAI-1), high mobility group box protein-1 (HMGB1), endothelial protein C receptor (EPCR) and soluble vascular cell adhesion molecule -1 (sVCAM-1) were all significantly higher in MM patients versus controls. In addition all the above were significantly reduced post treatment with melphalan-prednisone, bortezomib and lenalidomide. (392)

Contradictory evidence about the role of activated protein C resistance (APCR) in MM patients exists from earlier studies. Recently 99 MM patients were tested with second generation APC-Res Assay and confirmed a true incidence of APCR of 7.5%. A difference in VTE prevalence in patients with the alteration was also reported. (393) In contrast, Cini et al in 2010 found elevated APCR alterations but no association with increased VTE risk. (375)
Several single nucleotide polymorphisms (SNPs) in genes and pathways involved in drug metabolism and transport, DNA repair and cytokine balance were found to be associated with the risk of VTE in a group of MM patients. (29) In another group the only SNP found to be linked to increased VTE risk (OR 3.76) was in the NFκB1 gene. None of the patients in the

cohort carried polymorphisms of the common genetic variations associated with thrombosis (FVL and FIIG20210A) (364) Factor V Leiden and the prothrombin gene mutations seem to play a marginal role in the increased thrombotic risk associated with MM. (389, 394)

Thrombin generation (TG) is being increasingly studied by many groups who perform measurements at baseline, during treatment and in relation to VTE occurrence. Most groups have reported abnormal TG in multiple parameters of the assay compared to healthy controls. (395, 396) Data is variable and difficult to compare across studies as different TG assays have been used, different TG trigger concentration, phospholipid reagents etc. Crowley et al compared TG in MM patients, patients with MGUS and healthy controls and found endogenous thrombin potential (ETP) was lower in MM patients compared to healthy controls and Peak thrombin concentration was similar. Velocity index was higher, lag time shorter and time to Peak shorter in MM patients. (390) Ay et al in 2011 found thrombin peak concentration and ttPeak to be associated with VTE risk but not ETP. (238) Legendre et al in 2018 performed a pilot study in a few MM patients and assessed TG to find that it was attenuated compared to healthy controls with prolonged lag time and time to peak with decreased peak and ETP. (397) Leiba et al obtained blood samples from 36 MM patients (both NDMM and RRMM) and found significantly higher ETP and peak thrombin concentration in patients who developed VTE compared to those who did not. (372) Another group recently published data on 71 patient with MM and performed serial analysis of thrombin generation parameters during the first 4 cycles of treatment. TG parameters remained unchanged throughout treatment irrespective of treatment regimen but were significantly higher before cycle 2 and 3 for patient who received IMiDs. No association was determined between baseline levels of ETP, thrombin peak concentration or time to peak and VTE. (366) Dargaud et al recently published data on the potential role of thrombin generation assay (TGA) in the prediction of VTE risk in MM patients. (373) A total of 106 MM patients and 89 healthy adults were recruited on the study. TG capacity (Endogenous thrombin potential ETP) was higher in MM patients both in Platelet poor plasma (PPP) and platelet rich plasma (PRP). IN RPR TG was significantly higher in patients treated with lenalidomide compared to MM patients who did not receive IMiDs. TG capacity was significantly lower in patients who received LMWH or warfarin and aspirin had no impact on

TG in PPP but in the presence of platelets it exerted significant antithrombotic effect similar to that of LMWH suggesting a similar TG capacity when either LMWH or aspirin is used.

Bagratuni et al in 2013 studied single nucleotide polymorphisms (SNPs) related to perturbed endothelium in 200 consecutive unselected patients to assess how these may contribute to hypercoagulability. Twelve patients developed VTE (they were all receiving aspirin prophylaxis). None of these patients carried polymorphisms of the common genetic variations associated with thrombosis (FVL and FIIG20210A). The only SNP found to be associated with increased risk of VTE (OR 3.76) was in the NFκB1 gene. (364)

6.2.3 Treatment related Risk factors:

Treatment choice is a major determinant of VTE risk in MM patients. It is most likely the best understood in terms of risk stratification. VTE risk varies depending on treatment choice and combination from smaller than 2% to above 26%. Table 19 summarizes the most recent studies reporting VTE rates for different chemotherapeutic regimes.

The use of erythropoietin (EPO) is very common in MM patients and its association with increased VTE risk is well established. In a large multicentric observational study (MELISSE) multivariate analysis of 524 patients treated with IMiDs, the risk of VTE almost doubled with EPO use (10% versus 5%). (324) In another study, 604 NDMM patients were randomized to receive EPO and thalidomide versus thalidomide alone. Patients on EPO had a significantly higher incidence of VTE. (365)

In 1999 a VTE risk of 10% was reported in a group of NDMM patients who received Vincristine, Adriamycin and Dexamethasone (VAD). Multiple incidence reports have followed since. The improved outcomes in survival observed with the introduction of novel agents such as proteasome inhibitors (PIs) and IMiDs (thalidomide and its derivatives lenalidomide and pomalidomide) have been accompanied by a rise in VTE rates mainly due to the use of IMiDs.

Conventional therapy with Melphalan and Prednisone (MP) is associated with a low VTE risk of 1-2%. VTE rates seen with thalidomide monotherapy are not much higher; VTE risk is 3-4% in NDMM and 2-4% in RRMM patients. The addition of thalidomide to MP (MPT) raises that risk up to 17%. Reported risk of VTE is also increased to 8-25% with the addition of dexamethasone and to 35% when thalidomide is combined with anthracyclines. (375, 379, 382)

The VTE risk associated with lenalidomide (Len) monotherapy is not high, about 3% (398) In a meta-analysis on IMiD associated VTE risk, thalidomide plus dexamethasone was linked to a 1.3 VTE events per 100 patient cycles in NDMM and lenalidomide plus dexamethasone to 4.1. Rates were lower for RRMM patients. (231) In a trial which compared Len with low (40mg weekly) versus high (40mg on days 1-4, 9-12 and 17-20) dose dexamethasone the respective VTE rates were 12% and 26%. (399) Similar results were observed from other series combining Len with high dose dexamethasone. (400, 401) The rates are lower with Len maintenance post autologous stem cell transplant (ASCT) without thromboprophylaxis and one group reported 6% VTE rate during a median follow up of 45 months. (83) The associated VTE risk persists over time and does not decrease as the duration of exposure increases. (402) (403) Data on lenalidomide-associated VTE risk are presented more extensively in table 19. Fewer data exists on thrombotic risk linked to pomalidomide which is lower compared to lenalidomide but may reflect the current mandatory use of thromboprophylaxis. (404) Reported VTE rates vary depending on the dose of pomalidomide and range from 3-7% with 4 mg pomalidomide combined with dexamethasone to 0-6% with 2mg plus dexamethasone. (405) (406, 407) Contrary to the thrombogenic effect of IMiDs, the proteasome inhibitor bortezomib is associated with much lower rates of VTE. The SUMMIT and CREST RCTs report VTE rates of 0.6 and 2.7% respectively. The very low rates of VTE when bortezomib is used in association with drugs of known thrombogenic potential imply that bortezomib might mitigate their thrombogenic effects. (408)

Carfilzomib is a novel proteasome inhibitor and ASPIRE, a multicenter open-label trial, randomized patients to receive lenalidomide plus dexamethasone versus lenalidomide,

dexamethasone and carfilzomib. During cycles 1-12 of therapy, the VTE rate was 13% in the 3-drug arm versus 6% in the 2-drug arm despite mandatory use of thromboprophylaxis. More studies are required to determine whether carfilzomib is associated with a low risk of VTE, whether it has no effect on VTE risk or acts synergistically with IMiDs to increase VTE risk. (90) Increased VTE risk does not seem to be one of the adverse events linked to Elotozumab, Daratumumab or Ixazomib among the available approved drugs for MM patients. (409, 410) (411, 412) (89, 413)

The exact mechanisms underlying the IMiD- induced thrombogenic effect are not known. Association studies so far have hypothesized a role for increased cytokine levels, enhanced platelet aggregation and activation, cytokine mediated activated protein C resistance, increased vWF and VIII levels and procoagulant effect on endothelial cells through the increase in TF activity. Thalidomide has been also linked to a reduction in thrombomodulin as there is transient drop in thrombomodulin is seen during the first month of therapy which recovers over the next months.

Study	Regimen	Thromboprophylaxis	VTE rate
Carrier et al 2011(231)	Thalidomide		1.3 per 100pc
	TD -NDMM		4.1 per 100pc
	TD- RRMM		0.8 per 100pc
	RD -NDMM		0.8 per 100pc
	RD- RMM		0.7 per 100pc
Weber et al 2007 (414)	RD - RRMM (n=177)	Physicians discretion	14.7%
	placebo (n=176)		3.4%
Cini et al 2010 (375)	TD (n=246)	Warfarin	10.6%
	TD (n=19)	No thromboprophylaxis	86.2%
Palumbo et al 2012 (382)	T NDMM		3.5%
	T RRMM		<2%
	TD		8-26%
	MPT		17%
	VAD+T		58%
Fouquet et al 2013 (n=200) (398)	R-based regimens	ASA or LMWH	
	NDMM		9.4%
	RRMM		4.5%
Bagratuni et al 2013 (364)	R	ASA	6%
	R-NDMM		9.4%
	R-RRMM		4.5%
Dimopoulos et al 2007 (415)	RD- RRMM	none stated	11.4%
	placebo - RRMM		4.6%
Dimopoulos et al 2014 (403)	RD		12.7%
Rajkumar et al 2010 n=353 (399)	Rd NDMM		12%
	RD NDMM		26%
Rosovsky et al 2013 (400)	R- RRMM	yes	2%
	RD -RRMM	no	9%
Leleu et al 2013 (324)	R- RRMM		4.5%
Leebeek et al 2012 (359)	T		2%
	TD		7-26%
	MPT		12-15%
	R		4%
	RD		8.5%
Zonder et al 2010 (401)	R - NDMM	ASA	6%

	RD -NDMM	ASA	23.5%
Dimopoulos et al 2013 (416)	R-MP	ASA	3%
Stewart et al 2015 (n=792) (417)	KRD - RRMM	yes not specified	1 year rate 13%
	RD- RRMM	yes not specified	1 year rate 6%
Larocca et al 2012 (355)	R-MP +ASCT (n=342)	aspirin	2.27%
		enoxaparin	1.2%
	RD		11-15%
Bradbury et al 2017 (n=3838) (418)	CD-R NDMM	as per IMWG 2008	11.8%
	CD-T NDMM	as per IMWG 2008	11.8%
Hanaizi et al 2015 (419)	Pomdex RRMM		3.3%
Richardson et al 2014 (405)	Pomdex RRMM	yes	2%
Leleu et al 2013 (407)	Pomdex RRMM	ASA	7%
Paludo et et al 2018 n=50 (420)	PVD - RRMM	aspirin, LMWH, warfarin	10%

Table 19: Venous Thromboembolism with Different Anti-Myeloma Regimens
TD: Thalidomide plus dexamethasone; R: Lenalidomide; Rd: Lenalidomide plus low dose dexamethasone; RD: Lenalidomide plus high dose dexamethasone; NDMM: Newly diagnosed Multiple Myeloma; RRMM: Relapsed Refractory multiple myeloma; Pomdex: Pomalidomide plus dexamethasone; PVD: pomalidomide, velcade plus dexamethasone; ASA: aspirin; MPT: melphalan, prednisone plus thalidomide; R-MP: Lenalidomide, Melphalan and prednisone; ASCT: Autologous stem cell transplant; VAD+T: Bortezomib, Adriamycin, Dexamethasone plus Thalidomide; CD: cyclophosphamide- dexamethasone; KRD: Carfilzomib, Lenalidomide plus dexamethasone.

There are reports that thalidomide might alter the expression of protease-activated receptor 1 (PAR01) on the injured endothelium and regulate the expression of COX-2 or downregulate PU.1, a key transcription factor for granulocyte differentiation that leads to increased cathepsin G levels and higher VTE risk. (421) Platelet activation by thalidomide is likely to be one of the mechanisms by which it causes thrombosis. One study showed increased expression for PAC-1 (antibody that recognizes conformational change of the GPIIb/IIIa complex) after 4 weeks of thalidomide treatment. Individual immune response might affect the effect of thalidomide as immune modulation may lead to early clearance of

activated platelets. (422) High dose dexamethasone increases the P-selectin, vWF and FVIII levels (423) and doxorubicin seems to induce a procoagulant phenotype on endothelial cells and to increase the levels of plasma thrombin that is generated. (424)

Lenalidomide has been postulated to cause upregulation of cathepsin G, a potent platelet activator and in other studies levels of endothelial stress markers like ICAM and PAI-1 and VEGF were increased. Higher levels of P-selectin, fibrinogen and homocysteine following lenalidomide treatment have also been reported. An *in vitro* study of lenalidomide suggests that cell surface procoagulant activity (PCA) may be induced on endothelial cells by lenalidomide through TF-expression and exposure of protein S (PS). (425)

The protective effect of bortezomib is most likely due to the inhibition of the 26S proteasome, which is followed by inhibition of the NFκB transcription and affects several signaling pathways. The transient inhibition of platelet budding causes transient thrombocytopenia. Adenosine diphosphate (ADP) decreases platelet aggregation and results in platelet function inhibition. (426) (421) Zangari et al reported a significant decline in epinephrine and ristocetin induced platelet aggregation post bortezomib treatment and reduced expression of P-selectin on the platelet surface. (427)

6.3 Myeloma and Thromboprophylaxis

The data and literature on which IMWG, EMN and NCNN thromboprophylaxis recommendations for VTE prevention in MM patients have been reviewed by our group extensively. (428) (429) They are all on agreement that aspirin (80-325mg) is sufficient only for very low risk patients and that LMWH at prophylactic doses or warfarin with a target INR of 2-3 should be opted for in higher risk patients. Robust data from clinical trials are limited and therefore recommendations by expert groups have been developed also based on expert opinion. Important issues when considering the mode of thromboprophylaxis

includes effectiveness but also route of administration and patient convenience opting for the least intrusive method and safety in the context of thrombocytopenias, minimizing bleeding risk and impaired renal function all of which are very relevant in the context of MM.

The role of aspirin as an agent for thromboprophylaxis in MM patients has been questioned over the years and its use has been supported by data that demonstrate altered platelet function. The mononoclonal immunoglobulin might affect platelet adhesion and aggregation and IMiDs have been shown to exert their prothrombotic effects via enhancement of platelet activation. (382, 384, 399, 422) Another possible mechanism could be the inhibition of platelet aggregation via interference with the levels of circulating TNF and inhibition of NFκB.

One of the few RCTs ever to take place compared the use of aspirin 100mg once daily to enoxaparin 40mg once daily sc in 342 patients who received melphalan-prednisone (MP), then Lenalidomide and dexamethasone (Rd) and then autologous stem cell transplant and did not report significant differences in VTE rates between the two groups (2.27% in the aspirin versus 1.2% in the enoxaparin group). (355) In the Palumbo 2008 study in NDMM patients who received MP-Rd and aspirin 100mg, VTE rate was 4.8%. (356) In the Myeloma XI study patients between a lenalidomide or thalidomide based triplet combination (cyclophosphamide and dexamethasone) and per protocol thrombosis risk assessment was performed according to the IMWG 2014 guidelines. VTE rate was seen in 11.8% of patients and among them 31% were on aspirin, 44.1% on prophylactic LMWH and 9.2% on prophylactic LMWH and treatment dose warfarin. Direct comparison of effectiveness cannot be made as risk adjustment for VTE had taken place. (430) Two recent large reviews have added to the controversy of its use. Recently a systematic review of over 1126 patients VTE rate was 1.4% in patients on LMWH versus 10.7% in patients receiving aspirin demonstrating inadequate prophylaxis in patients considered to be low risk. (431) In another large retrospective review of 4892 patients with MM, 586 patients developed VTE and when adjusting for risk factors such as IMiD use and past history of VTE, aspirin did not reduce the risk of VTE enough to justify its role. (432) Careful evaluation of the above data is

required however as IMWG guidelines justify its use only in very low risk patients. The group of Swan et al suggest that aspirin use should be avoided as thromboprophylaxis during the first 6 months of treatment in NDMM who receive IMiDs but a switch to aspirin could be considered in patients who are in remission after 6 months. (433) Suggesting the optimal dose is not possible but most clinicians and studies favor 100mg. The hypothesis that low doses may be more effective due to selective inhibition of thromboxane biosynthesis has not been proven in myeloma patients. [32] Another RCT compared ASA and fixed low dose warfarin (1.25mg/day) to LMWH (Enoxaparin 40mg/day) as agents of VTE prevention in 667 NDMM patients who received thalidomide. The rate of VTE was 6.5% overall; 6.3% in ASA group, 8.2% in warfarin group and 5% in LMWH group. (354) Zangari reviewed phase 3 trials with novel combination regimens and reported a marked decrease in VTE incidence (15% from 34%) was observed with the introduction of LMWH in patients treated with thalidomide. (408) Low dose warfarin was shown to decrease VTE rate to 11.6% versus 26% in the group that did not receive thromboprophylaxis in patients receiving lenalidomide and dexamethasone prior to ASCT.(379) Prophylactic doses of LMWH are used and are considered more appropriate than warfarin for cytopenic patients. Limiting factors include need for self –injection and compliance, cost and need to adjust for impaired creatinine clearance. Warfarin has the highest bleeding risk and is most unpredictable due to interpatient variability in pharmacokinetic and pharmacodynamics responses. Optimal dosing is often iteratively guided by INR but in this population fixed low dose warfarin of 1-1.25mg has been studied.

DOACs are either inhibitors of factor Xa (apixaban, rivaroxaban, edoxaban, betrixanab or IIa (dabigatran) and their use has become increasingly favorable in recent years given their user friendly route of administration and lack of need for monitoring at standard doses compared to warfarin. There is increasing evidence for their use in the treatment of VTE for patients with malignancy but their role in thromboprophylaxis remains unclear yet and none is currently licensed for this use. (312) As discussed already DOACs are lisenced for treatment of CAT and have shown comparable efficacy and improved safety compared to the oral alternative warfarin in patients with cancer.

A retrospective review assessed safety and efficacy of DOACs (dabigatran, rivaroxaban or apixaban) versus warfarin in patients on IMiD-based regimens and reported 4 non-major bleeds in the DOAC group versus six in the warfarin group. (434) Another group compared VTE event rate in prior and post 2014 and the introduction of a policy change in their center to use apixaban 2.5 mg twice daily as routine thromboprophylaxis for patients on IMiDs. Prior to 2014, a VTE rate of 20.7% was reported in patients on aspirin and 7.4% in patients on LMWH compared to no VTE events after 2014 within 6 months of treatment initiation. (435) The ongoing single arm phase IV study (NCT02958969) will evaluate prospectively the safety and efficacy of apixaban for primary VTE prevention in MM patients. The primary objective is to assess VTE occurrence within 6 months in patients who receive IMiD based therapy. (436) Data from the pilot study of this clinical trial were presented at ASH this year. (325) Fifty patients received apixaban 2.5 mg orally twice daily and were prospectively monitored for 6 months (excluding patients requiring therapeutic anticoagulation or with a prior history of VTE). The median number of prior lines of therapy was 2, 58% of patients were on lenalidomide containing regimens, 42% pomalidomide containing and 66% were receiving IMiDs post-autologous stem cell transplant as consolidation or maintenance therapy. At interim analysis at 3 months no patients had experienced major hemorrhage or VTE. (325) Results from another pilot study were recently released by Pegourie et al which also aimed to assess the effectiveness and safety of apixaban in MM patients treated with IMiDs. Two events of VTE were reported in 140 patients receiving apixaban 2.5mg twice daily over 6 months. (437) RCTs are needed designed specifically to compared the use of DOACs, LMWH and warfarin as means of VTE prevention in MM patients.

The advantages of DOACs over heparins is the oral route of administration and over warfarin the lack of need for monitoring. The oral route is however affected by changes in gastrointestinal absorption. Impaired renal function, co-existing thrombocytopenias and frailty of older age are all very relevant considerations for all modes of thromboprophylaxis in MM patients. LMWHs and DOACs are contraindicated in patients with GFR < 30ml/min. Recommendations and dose adjustment criteria in cases of renal impairment differ across each DOAC. (438) Data for patients with end-stage renal disease and on dialysis are limited. Most authors would recommend use of unfractionated heparin and vitamin K antagonists or

LMWH adjusted to anti-Xa levels for patients with GFR <30 ml/min for VTE treatment. Regarding thromboprophylaxis data is scarce and decisions should be made on a case-by-case basis. (439) The empirical cut off of 50000/mm^3 platelet count is used by most clinicians for the administration of full LMWH administration (440) and most clinicians will half the dose when platelet counts range from 49000 to 30000/$mm^{3.}$ Empirical practice currently is to consider use of DOACs for thromboprophylaxis safe when platelet counts are > 75-80.000mm^3. The cut-off is lower at >50000/mm^3 when the indication is treatment of venous thromboembolism. (441) Drug-drug interactions also require attention with the use of DOACs as they are substrates of P-glycoproetin and rivaroxaban and apixaban of cytochrome P450. (442-444) Among anti-myeloma agents (except dexamethasone) no agent is a strong inhibitor or inducer of these pathways and current regimens do not raise significant concerns regarding interactions.

Further data from prospective RCTs that assess use of DOACs in MM patients are required. We need data for use in the setting of the newly diagnosed and the relapsed/refractory patient with MM, data for patients on IMiDs but also on non-IMiD containing regimens. Direct comparison against LMWH for higher risk patients and against aspirin for lower risk patients would provide robust clinical data to guide thromboprophylaxis management.

6.4 Risk assessment models for Venous thromboembolism in Multiple Myeloma

The IMWG recommends thromboprophylaxis for patients with NDMM on IMiDs for the 4-6 first months of treatment or for as long as the risk of thrombosis remains high and the choice of prophylaxis medication depends on baseline risk stratification. Patients with 0 or 1 risk factors should receive aspirin (80-325 mg) and patients with 2 or more risk factors prophylactic dose LMWH. (445) Risk factors that should be taken into account can be seen in Table 20. A clear recommendation for MM patients on non-IMiD based treatment is lacking. IMWG, EMN and NCCN guidelines are all based on the limited data available from RCTs. The extent to which they are being applied in everyday clinical practice is questionable

according to some recent reports, as clinicians tend to opt for thromboprophylaxis based more on personal perception of thrombotic risk. (363) (430) Even when recommendations are being applied residual VTE rates remain significant pointing to the need for optimization of current guidelines.(342, 430) More sensitive risk stratification tools are required to reflect more accurately all aspects of the procoagulant environment that exists in patients with MM.

There is therefore need for clinical scores that can assess thrombotic risk sensitively for individual MM patients. The Myeloma Clot score (MSC), the IMPEDE and the HAS-RISC scores were recently presented but have not been clinically validated. (446-448) These include many of the risk factors (patient-specific, disease-specific and treatment-specific) identified up -to date which are also included in the risk assessment proposed by the IMWG but allow for more appropriate weighting and include also negative weighting for factors that are protective against thrombosis. These attempts are very encouraging and hopefully future versions of risk assessment models will allow accurate determination of the risk of thrombosis in this very complex and heterogeneous population. Some groups hold the belief that improvement of risk model sensitivity requires the incorporation of coagulation biomarkers. In that context, there are ongoing efforts to investigate the complex coagulation environment of MM patients and to identify generic biomarkers that reflect VTE risk. (342) (372, 397) It should be noted that none have been validated or incorporated in a clinical model yet. Also to be of use such a biomarker should be easily measured in every day clinical practice and not require laboratory expertise or be associated with high costs.

Patient Related (1 point)
BMI>30, Age, Race Personal or family history of VTE Comorbidity: cardiac, Diabetes mellitus, renal impairment, liver impairment, chronic inflammatory disease, COPD, immobilization Recent surgery (<6weeks), general anesthesia or trauma Central venous catheter Acute infection Hospitalization Thrombophilia/ blood clotting disorders Hormone replacement therapy or tamoxifen Blood clotting disorders (anti-thrombin deficiency)
Disease Related (1point)
Myeloma diagnosis Hyperviscosity
Data from studies on potential disease related prothrombotic mechanisms – not incorporated into risk assessment *Monoclonal component Related:* • *Lupus Antibody Coagulant -like activity (379)* • *Altered properties of fibrin fibers and fibrin polymerization (380)* • *Enhanced platelet adhesion and dysfunction (382-384)* • *Anti-thrombin, Protein-C and Protein-S antibodies (360)* • *Acquired Activated protein C resistance (393)* *Hypercoagulability related:* • *Tissue factor (TF), fibrinogen, factor VIII (FVIII) and von Willenbrand Factor (vWF) + Increased levels of pro-inflammatory cytokines (360, 382-384)* • *Increased levels of tissue factor- derived microparticles) (252, 287, 449)* • *Downregulation of thrombomodulin, endothelial protein C receptor (EPCR), APC (activated protein C), and tissue plasminogen activator (t-PA) (360, 376, 384, 450)* • *Increased plasminogen activator inhibitor levels (PAI-1) (371)* • *Single nucleotide polymorphisms (SNPs) and the NFκB1 gene (364)* • *Increased endothelial tissue factor expression (451)* • *Increased cell surface phosphatidylserine expression (451, 452)* • *Changes in thrombin generation parameters (373, 397)*

Myeloma Therapy Related
IMiD in combination with (2 points)
· High-dose dexamethasone (>480mg/month)
· Multi-agent chemotherapy
· Doxorubicin
IMiD alone (1 point)

Table 20: Risk factors of VTE in patients with Multiple Myeloma. Abbreviations: LAC: lupus anticoagulant, PC: protein C, PS: protein S, TF-MP: tissue factor microparticles, VWF: von Willenbrand Factor, APC: activated protein C, t-PA: tissue plasminogen activator, PAI-1: plasminogen activator inhibitor, SNP: single nucleotide polymorphisms, IMiDs: immunomodulatory drugs. Multi-agent chemotherapy: IMiDs with dexamethasone or anthracyclines or more than two other agents, any combination therapy that includes corticosteroids and more than 3 agents like cisplatin, etoposide, cyclophosphamide.

There is an ever increasing need for RCTs designed to compare modes of thromboprophylaxis in MM patients based on clear cut risk stratification criteria in order to provide robust data for optimum management of this significant complication in MM patients. Advances in the understanding of how coagulation pathways are altered in the context of myeloma are much anticipated.

Recently, some groups have proposed clinical scores for the assessment of VTE risk specific to MM patients. Sanfilippo et al developed the IMPEDE score by using 4.446 patients diagnosed with MM within the Veterans Administration Central Cancer Registry (n=4446) as a derivation cohort and the SEER-Medicare (Surveillance, Epidemiology, and End Results) database to validate the score derived (n=4,256). The final model of the IMPEDE score includes the following: : IMID 3 points, BMI 1 point, Pathologic fracture pelvis/femur 2 points, EPO 1 point, Dexamethasone (High-dose 4 points, Low-Dose 2 points)/Doxorubicin 2 points, Ethnicity/Race= Asian -3 points, history of VTE 3 points, Tunneled line/CVC 2 points), use of therapeutic anticoagulation (-5 points) with warfarin or low molecular weight heparin (LWMH) and use of prophylactic LMWH or aspirin (-2 points). Three risk groups were identified based on the score and VTE rates were 3.1% for low risk (scores ≤ 3), 7.5% for intermediate risk (score of 4-6) and 13.3% for high risk (≥ 7 score). (453)

Li et al used the SEER-Medicare (Surveillance, Epidemiology, and End Results) and the Veterans Health Administration (NVH) databases to assess the performance of the NCCN Guidelines in each database and then developed and validated a RAM for MM patients who receive IMiDs. The derivation cohort from the SEER database included 2397 patients with MM out of which only 13% received anticoagulation (11% warfarin, 1% LMWH, 1% DOAC). VTE occurred in 14.4% of patients with 8.3% of the events occurring during the first 12 months of continuous IMiD exposure. In the validation VHA cohort (most patients were male), anticoagulation was administered in 21% of patients and VTE occurred in 9.4%. The SAVED score combines 7 weighted clinical factors (with point assignment) surgery within 90 days (+2), Asian race (+3), VTE history (+3), age ≥80 years (+1) and dexamethasone dose (+2 for high, +1 for standard). Patients with ≥ 2 were considered high risk and patients with score <=1 were considered low risk. Incidence of VTE at 3 and 6 months was 7% and 12% in the high-risk group, respectively, versus 4% and 7% in the low-risk group, respectively. The score was externally validated using the VHA cohort. (454)

Our group recently proposed an algorithm for VTE prevention on the approach of thromboprophylaxis of the MM patient based on current established and emerging data (figure 2) (428). Risk stratification should currently be based on risk factors listed in table 20. Very low risk patients (no risk factors) should receive no thromboprophylaxis. Low risk patients who have no risk factors but receive IMiD based therapy (not high risk combinations) or have one other disease or myeloma related risk factor should receive aspirin 100 mg qd or prophylactic dose of a DOAC although this is not a licensed use and data should be awaited. High-risk patients are patients with 2 or more disease or patient related risk factors or patients on IMiD-based high risk treatment regimens. LMWH heparin at prophylactic doses or warfarin with a target INR 2-3 should be administered or DOAC at prophylactic doses although again they are not licensed for this use and data from RCTs should be awaited. Finally very high risk patients with more than 3 risk factors which include high risk factors such as previous history of VTE, known thrombophilia or IMiD-containing regimen combine with high dose dexamethasone or multi-agent chemotherapy, should also be considered for therapeutic doses of LMWH or warfarin with target INR at 2-3 or DOACs

(RCT data awaited). The patient-profile, renal function, bleeding risk, frailty and preference should all be taken into account. Thromboprophylaxis should be administered for 4-6 months post treatment initiation or for as long as the patient is considered to be at risk of thrombosis. Our group favors the use of LMWH over warfarin although this is not based on clinical data.

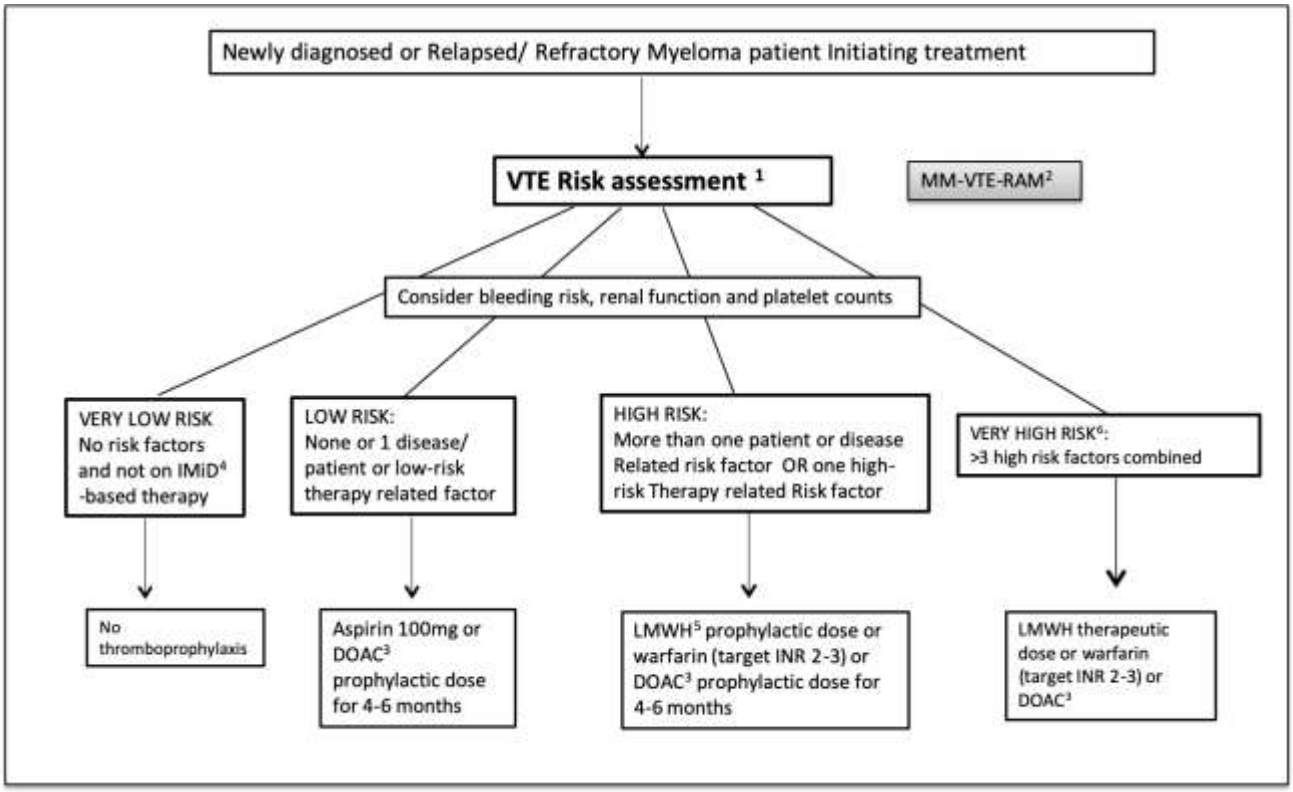


Figure 2: Algorithm for choice of thromboprophylaxis in patients with MM initiating treatment

[1] VTE risk assessment based on Table 1 Risk factors

[2] MM-VTE-RAM: Multiple Myeloma – Venous thromboembolism – Risk assessment model: to be developed and incorporated in the future in the risk stratification process

[3] DOAC: direct oral anticoagulants: Note that they are not licensed for this use and data from RCTs should be awaited before incorporation into clinical practice

[4] IMiD: immunomodulatory drugs: Lenalidomide, Thalidomide and Pomalidomide

[5] LMWH: low molecular weight heparin

[6] More than 3 risk factors which include high risk factors such as: previous history of VTE, IMiD in combination with high dose dexamethasone and known thrombophilia

THESIS

1. Purpose and importance of the proposed research paper

Venous thromboembolism (VTE) remains one of the common complications in patients with multiple myeloma (MM). (336) Approximately 10% of patients with newly diagnosed MM (NDMM) will develop VTE during their disease course. (337-339). Most published data support an inferior overall survival in MM patients with VTE (349) (350) (338). In the era of novel agents and the ever increasing armamentarium of treatment options outcomes have dramatically improved for these patients. Longer survival however requires tools for the effective management of the adverse effects associated with the novel agents used. VTE risk in MM is treatment-dependent to a considerable extent as the 2% VTE rate reported for melphalan and dexamethasone increases up to 26% when Immunomodulatory (IMiDs) agents are used in combination with multiagent chemotherapy regimens and high dose dexamethasone.

The International Myeloma Working Group (IMWG) 2014 statement (445) and the European Myeloma Network Guidelines in 2015 (91) proposed a risk stratification algorithm and relevant thromboprophylaxis guidelines for MM patients who receive IMiDs. These were later incorporated and into the NCCN guidelines for the management of VTE in cancer patients. (455) The recommendations are based to some extent on the limited robust clinical data available on the use of aspirin, LMWH) and warfarin but also on expert opinion. (354-356, 456, 457) In summary they suggest for MM patients on IMiDs low dose aspirin when one or none VTE risk factors are present and prophylactic LMWH or dose-adjusted therapeutic warfarin for 2 or more risk factors for 4-6 months at the time of treatment initiation or at disease relapse. There is lack of clear guidance on thromboprophylaxis for MM patients who are not on IMiD - containing regimens.

These guidelines have become available since 2014, but the risk of residual VTE remains high as demonstrated in recent reports. (342) (363, 420) Subanalysis from the Myeloma XI study demonstrated that the available risk stratification algorithm identified only 55% of patients who later developed VTE as high risk at baseline. (430) There is also inconsistency

in the application of existing recommendations in clinical practice and data show physicians tend to apply thromboprophylaxis based mostly on clinical experience. (458, 459) The current algorithm is therefore suboptimal as it fails to effectively identify high-risk patients and guide thromboprophylaxis appropriately. There is no validated risk stratification tool for VTE risk assessment in MM patients at present. In addition the optimal method of thromboprophylaxis has not been determined as there is lack of clinical trials that have attempted to address this clinical question.

VTE risk assessment in MM patient is a challenging endeavor as the thrombogenicity observed in myeloma is multifactorial. Risk factors are not only treatment related and patient specific but linked to the disease pathology itself. (360, 361) There is crosstalk between the monoclonal plasma cell, the bone marrow microenvironment and components of coagulation which results in the hypercoagulable environment observed. Up to date there is limited understanding of the complex interplay of all the involved risk factors and their effect on pathways of coagulation. Delineating the coagulation profile of the MM patient will allow the identification of biomarkers of cellular and plasma hypercoagulability for the incorporation in a RAM together with other risk factors. Given the data that report higher than baseline VTE risk in the MM precursor states (MGUS and SMM) there is value in studying the coagulation profile throughout the continuum of this unique plasma cell dyscrasia.

The first step towards minimizing VTE rates is therefore the development of effective and validated risk stratification tools in the form of risk assessment models (RAM) that can capture all aspects of the pro-thrombotic environment that exists in MM patients. In recent years a lot of focus has been placed on the development of RAMs for cancer related VTE risk. It has been proposed that the incorporation of biomarkers of blood hypercoagulability and endothelial cell activation might increase the sensitivity of RAMs for VTE risk identification.(238) Some groups have therefore turned their attention and efforts in identifying relevant biomarkers in MM patients that could contribute towards optimizing thromboprophylaxis. Once the optimal tool has been developed the second step is to choose the appropriate, most safe and effective tool for thromboprophylaxis; the right

agent for the right patient, for the right amount of time. There is very limited data to support best-clinical practice in the application of thromboprophylaxis in MM. There are no head-to-head comparisons of different agents of thromboprophylaxis and there is lack of data that supports what is the optimal duration and timing of treatment.

In the context of the need for risk stratification improvement and targeted thromboprophylaxis our group has designed and initiated the prospective, observational study ROADMAP-MM (PROspective Risk Assessment anD bioMArkers of hypercoagulability for the identification of patients with Multiply Myeloma at risk for Cancer-Associated Thrombosis) (ClinicalTrials.gov identifier NCT03405571) The aim of this ongoing study is to identify in NDMM patients relevant biomarkers of hypercoagulability, variables related with MM and clinical predictors of VTE risk that could be combined to risk stratify MM patients and guide thromboprophylaxis. The development of a RAM which would then be validated would be used in a randomized controlled clinical trial to study the effectiveness of its use.

2. METHODS

2.1 Study design

The study was an investigator initiated prospective non-interventional trial and event driven.

Objectives of the study:

- The study of cellular and plasma hypercoagulability in patients with myeloma.
- To determine MM patients at high risk for VTE who are eligible for thromboprophylaxis.

Secondary objectives:

To identify in NDMM patients relevant biomarkers of hypercoagulability, variables related with MM and clinical predictors of VTE risk that could be used in combination in a RAM to risk stratify MM patients and guide thromboprophylaxis.

Hypothesis:

1.The biological profile of the coagulation mechanisms in NDMM will differ from that of healthy controls.

2. Patients who will be classified as high risk according to the ROADMAP-MM-CAT RAM will be at higher risk of VTE.

3. The prospective validation of the RAM will allow to identify patients eligible for long-term thromboprophylaxis with intermediate dose of tinzaparin

Study end-points:

Primary end-point: the occurrence of symptomatic VTE (DVT and/or PE) or superficial venous thrombosis of the lower or upper limb, central vein catheter thrombosis or venous thrombosis of rare localization. Venous thrombosis has to be confirmed with any of the following assays: echo-Doppler, CT or MRI angiography, or scintigraphy or CT scan. Occurrence of asymptomatic venous thrombosis found during routine imaging for staging will also be included in the primary end point. Combined end-point including all the above types of venous thrombosis will also be evaluated. Patients will be followed up for 1 year following study enrollment.

Secondary end-points: mortality, major bleeding (according to the ISTH criteria), cancer evolution and morbidity during follow-up.

Inclusion criteria: Newly diagnosed, treatment naïve symptomatic patients with MM (based on 2014 IMWG Criteria) (1) were diagnosed or referred to the Department of Clinical Therapeutics (Alexandra Hospital, Athens, Greece).

Other patients with plasma cell dyscrasias were also enrolled in the study including:

- Patients with MGUS
- Patients with asymptomatic MM

All patients provided written informed consent and all patients received anti-myeloma treatment according to institutional practice.

Exclusion criteria

The exclusion criteria more analytically were: age younger than 18 years, life expectancy less than 6 months, ongoing pregnancy, major psychiatric disorders, recent (<6 months) episode of VTE or acute coronary syndrome, active anticoagulant treatment (for any indication), scheduled open elective curative surgery under general anesthesia for abdominal or pelvic or lung cancer, hospitalization due to stroke or acute coronary syndrome or congestive heart failure or acute respiratory failure. Eligible patients had not undergone any surgery in the preceding 3 months.

Procedures:

- Screening and informed consent was be taken by the investigator
- Plasma samples were be prepared and stored at the investigation center under the responsibility of the Investigator.
- Samples were centralized to the core laboratory at Thrombosis Center, Service d'Hématologie Biologique, Tenon University Hospital, Paris.
- A combined data base of clinical and laboratory data was constructed.

Timepoints of planned procedures:

At inclusion, at 3,6 and 12 months the following procedures will be performed:

- Clinical data are recorded in a pre-specific clinical research form (CRF)
- Doppler Ultrasound of the lower limbs is performed
- Blood samples are obtained

2.2 Clinical research form (CRF) data and definitions for key predictors for VTE

The CRF included an exhaustive list of previously validated risk factors for VTE among other clinical and disease data. The CRF assessed the status of the disease, the ongoing treatments, the devices and the values of hemogram and laboratory parameters of liver and renal functions measured within one week prior to enrollment. The comorbidities and VTE risk factors non-related to the MM were defined as follows: renal function was considered as normal if the estimated creatinine clearance rate using Cockcroft-Gault formula was ≥ 60 mL/min/1.73 m^2. Liver impairment was defined as transaminase increase 2-fold higher than the upper normal level. The body mass index (BMI) at the day of the assessment was stratified into three groups: normal weight (BMI lower than 25), overweight (BMI higher or equal to 25 but lower than 30) or obese (BMI greater or equal to 30). The predictors "hyperlipidemia", "hypertension", "diabetes", "personal history of acute coronary syndrome", "stroke" and "peripheral artery disease" appeared individually in the CRF, were assessed at the inclusion and refer to objectively diagnosed conditions according to the respective diagnostic criteria. Total bed rest with bathroom privileges for > 3 days was evaluated when occurred within one month prior to the inclusion in the study. The predictor "pulmonary disease" includes any active pulmonary disease (except cancer) requiring treatment and at least within one month prior to inclusion in the study. The predictor “cardiovascular risk factor” was determined if the patient had any of the following; hypertension, hyperlipidemia, ischemic heart disease, smoking, an arrhythmia or diabetes. Patients we categorized as low (0-1 risk factors), medium (2-4 factors) and high risk (5-6 risk factors) for cardiovascular disease. The "hospitalization" was defined as hospitalization for any non-surgical reason occurring within the last 3 months before assessment. Performance status (PS) was assessed according to the ECOG classification. At diagnosis patients were classified according to the International staging system for multiple myeloma as stage I, II or III. Patients were classified as having high risk cytogenetics at diagnosis if bone marrow fluorescence in situ hybridization analysis was positive for t(4;14), t(14;16) or del17q cytogenetic abnormalities. The presence of lytic bone disease was assessed with whole body computerized tomography (WBCT) scans. Magnetic resonance imaging (MRI) of the thoracic and lumbar spine was used to assess the pattern of bone marrow infiltration (normal, focal pattern, salt-and-pepper or diffuse).

2.3 VTE confirmation and assessment:

The primary study end-point was symptomatic VTE, objectively confirmed by at least one of the following methods: color Echo-Doppler, computerized tomography, magnetic resonance imaging angiography, scintigraphy or computerized tomography scan.
Patients were routinely assessed for DVT with systematic compression ultrasound of the lower limbs at inclusion. A systematic compression ultrasound examination was performed by one operator, unaware of the history and treatment assignments, at the following time-points: baseline, at 6 months, at 1 year/ end of the study and at the event. All compression ultrasound examinations will be digitally recorded for further adjudication if needed.

Symptomatic VTE included deep vein thrombosis (DVT), pulmonary embolism (PE) or both (DVT and PE), superficial vein thrombosis located at distance of less than 3 cm from the saphenofemoral junction (SVT), central venous catheter (CVC) thrombosis or upper limb vein thrombosis (not related to the CVC) or vein thrombosis of rare localization (i.e. splanchnic vein or cerebral vein thrombosis). Symptomatic VTE had to be documented, by at least one of the following methods: color Echo-Doppler, computerized tomography, magnetic resonance imaging angiography, scintigraphy or computerized tomography scan. The investigators confirmed the occurrence of VTE by analysis of the patient's medical files taking into consideration the results of the imaging methods and the administration of therapeutic doses of anticoagulant by the treating physician. Patients with incidental VTE were recorded but not included in the analysis since research for this form of thrombosis has not reached definitive conclusions regarding the need to treat with anticoagulant therapy.

2.4 VTE risk assessment algorithm and thromboprophylaxis

VTE risk assessment and choice of thromboprophylaxis post enrollment in the study was along IMWG 2014 recommendations (91, 356, 445). (table 21) High risk patients (IMiDs plus >1 VTE risk factor) received tinzaparin 4.500 anti-Xa IU sc od, moderate risk patients (IMiDs

plus no risk factors or non-IMiDs with moderate risk for VTE) received aspirin 100mg po od and low risk patients (no or very low risk plus non-IMiD therapy) did not receive any thromboprophylaxis. The duration of thromboprophylaxis depended on treatment duration and type. Thromboprophylaxis choice was not considered to be an intervention as it is part of standard clinical care provided by the institution for this population.

<table>
<tr><th colspan="2">MULTIPLE MYELOMA RISK FACTORS FOR VTE SCORE</th></tr>
<tr><td>Individual risk factors (1 point each)</td><td></td></tr>
<tr><td>• Obesity (BMI >=30kg/m2)
• Previous VTE or family history of VTE
• Central venous catheter or pacemaker
• Comorbidity:
o Cardiac disease
o Chronic renal disease, diabetes
o Acute infection
o Liver impairment, Chronic inflammatory disease
o COPD
• Recent surgery (<6 weeks), trauma, any anesthesia
• Erythropoetin
• Thrombophilia / blood clotting disorders
• Immobility (PS>1)
• Hormone replacement therapy</td><td>Low risk = 0 points

Intermediate Risk= 1 point

High risk >1 point</td></tr>
<tr><td>Myeloma related (1 point each)</td><td></td></tr>
<tr><td>• Hyperviscosity
• New diagnosis</td><td></td></tr>
<tr><td>Treatment Related</td><td></td></tr>
<tr><td>• Multi-agent chemotherapy (1 point)
• IMiD alone or with low dose dexamethasone (1 point)
• IMiD + high dose dexamethasone (or doxorubicin or multi-agent chemotherapy (2 points)</td><td></td></tr>
</table>

Table 21: VTE Risk assessment algorithm and scoring at inclusion for patients according to the International Myeloma Working Group 2014 Guidelines

So the cohort of multiple myeloma patients was be composed by three pre-specified groups

- Low risk (O points) Group MM-No: patients with MM not receiving any antithrombotic treatment
- Intermediate risk (1 point) Group MM-ASA : patients with MM on treatment with aspirin
- High risk (> 1 point) Group MM-LMWH: patients with MM on treatment with usual prophylactic doses of tinzaparin

2.5 Blood sample collection and biomarker assessment

At inclusion and at the prespecified timepoints and according to the established practice of the investigation centers and the standardized institutional practice for laboratory monitoring of the patients, blood samples will be obtained after atraumatic vein puncture according to standardized procedure, in order to assess biomarkers of hypercoagulability. No additional blood samples to those routinely taken, will be required. The tests required for the present study will be performed using the residual blood from that required for routine laboratory tests.

Blood samples were routinely obtained by atraumatic antecubital venipuncture at baseline and collected in Vacutainer® tubes (5 ml tubes, containing 0.109 mol/L trisodium citrate - 1 volume trisodium citrate to 9 volumes blood). Antecubital vein puncture will be performed using 21G needles, or blood will be withdrawn from a recently (within 2-3 minutes) placed peripheral venous catheter (20G). Within 30 minutes after vein puncture samples were used to extract platelet-poor plasma (PPP) by double centrifugation at 2000 g for 20 minutes at room temperature and plasma aliquots were stored at -80 °C until assayed.

- **Procoagulant phospholipid-dependent clotting time *(Proag-PPL)*** was measured with STA® Procoag-PPL, according to the manufacturer's instructions (460, 461).
- ***Thrombomodulin activity*.** Plasma levels of thrombomodulin activity (TMa), were measured with a home-made test on the STA-R analyser (Diagnostica Stago, Asnières, France) as described elsewhere.
- The levels of factor **VIIa** (Staclot® VIIa-rTF), **factor V (FV), antithrombin (AT), fibrin monomers (FM)**, **free Tissue factor Pathway inhibitor (TFPI)** and **D-Dimers** were

measured with commercially available assays according to the manufacturer's instructions, on a STA-R® analyzer. The assays and the analyzer were purchased from Diagnostica Stago, Asnières France.

- **Tissue Factor activity (TFa)** in PPP was measured as previously described (460, 462, 463). The inter- and intra-assay coefficients of variation were 7% and 5%, respectively.
- **Plasma levels of P-Selectin and heparanase** were measured with ELISA Kits from Cusabio Biotech (CliniSciencies, France) and R&D Systems (Lille, France) respectively.
- **Thrombin generation assay in plasma**: Thrombin generation in platelet-poor plasma (PPP) was assessed using the Calibrated Automated Thrombogram assay (CAT®, Diagnostica Stago, France) as described by Hemker et al. Briefly 80 µl of PPP was added to 20 µl of PPP-reagent 5 pM® (Thrombinoscope b.v., Maastricht, Netherlands), that is a mixture of TF (5 pM final concentration in plasma) and phospholipids (4 µM final concentration in plasma). Each patient's plasma was studied in duplicate. In a third well, PPP reagent 5 pM® was replaced with the same volume of Thrombin Calibrator® (Thrombinoscope bv, Maastricht, Netherlands) to correct thrombin generation curves for substrate consumption and the inner filter fluorescence effects. Thrombin generation was triggered with a 20 µl solution containing $CaCl_2$ (16.7 mM final concentration) and the fluorogenic substrate Z-Gly-Gly-Arg-AMC (417 pM final concentration). Fluorescence was measured using a Fluoroscan Ascent® fluorometer (ThermoLabsystems, Helsinki, Finland). Acquisition of thrombin generation parameters was performed using the appropriate software (Calibrated Automated Thrombogram® bv, Maastricht, Netherlands). The following parameters of thrombogram were analyzed: (a) lag-time that indicates the initiation phase of thrombin generation, (b) time to reach maximum concentration of thrombin (ttPeak), (c) maximum concentration of thrombin (Peak), d) mean rate index (MRI) of the propagation phase of thrombin generation calculated by the formula: Peak/ (ttPeak – lag-time) and expressed in nM/min and e) endogenous thrombin potential (ETP) that shows the integral enzymatic activity of thrombin. Assay specifications have been published elsewhere. (464-466).

2.6 Ethical considerations

The study was conducted according to the principles of the last Declaration of Helsinki and in accordance with the Medical Research Involving Human Subjects Act (WMO) and other regulations, guidelines and acts. No benefits can be expected for individual patients who participate in this study. However, more information on this subject can help in the identification of patients with the highest risk of venous thrombosis. These patients might in the future benefit from prophylactic anticoagulants. If knowledge on this subject becomes incorporated into patient care, it will be in the same group of patients who participate in the present study. Results from this study, i.e. microparticle activity, did have an influence on regular patient care, because of its experimental character. Risks were not to be expected, because of the observational character of this study. The burden of the study is low, because it only includes a single blood withdrawal. Each patient has to make his or her own comparative assessment and decide on a voluntary base. The Medical Ethical Committee has given us dispensation from the obligation to provide insurance.

2.7 Administrative aspects:

All investigators had have access to the research data in a coded form. Patient data was coded and the code was stored and taken care of by the responsible investigator. Patient material was be stored in a coded form.

2.8 Healthy controls:

Biomarkers measured in the cohort of MM patients were compared against values previously measured in a group of healthy individuals with similar age and sex distribution, not taking any medication for at least one month before blood sampling. The normal values of the studied biomarkers were defined in the control group and were compared to the

corresponding normal reference range used by our laboratory. These normal ranges have been established according to the requirements for the good quality of laboratory practice by performing the tests in healthy individuals representative of the general population regarding age, sex, ethnicity and BMI.

2.9 Statistical analysis

A power calculation is difficult to make because this study concerns a pilot study. The results of the tests will be compared using the Student t-test or a non-parametric test where appropriate. Continuous variables are described as mean ± standard deviation and categorical variables as frequency and percentage. In view of the deviation from normality (as evidenced by the Shapiro-Wilk test), the comparison of biomarker levels between MM patients and healthy individuals was performed using the Mann-Whitney-Wilcoxon text for independent samples. Concerning the intercorrelations between biomarkers in MM patients, Spearman's rank correlation coefficients were estimated. At the univariate analysis the level of statistical significance was set at 0.05. Regarding the associations between VTE and biomarkers, the latter were converted to binary variables through Receiver Operating Characteristic (ROC) curve analysis; the selection of cut-off levels was based on the maximization of Youden's index. Subsequently, multivariate logistic regression analysis was performed with VTE as the dependent variable; biomarker variables proven significant at the univariate logistic regression analysis were examined as possible independent variables. Using a stepwise procedure, at the final multivariate logistic model all variables with *p*-value less than 0.10 were retained; the area under the ROC curve (AUC) was estimated to describe the fit of the multivariate model. Data were analyzed using the STATA/SE version 13 statistical software (Stata Corp., College Station, TX, USA).

3. RESULTS:

Up to date 480 patients have been enrolled in the study from June 2014 up to June 2019. Out of the 380, data and sample analysis has been performed in 144 Multiple myeloma patients, 80 SMM patients and 54 MGUS patients. Data on patients with symptomatic MM were published in 2018. (342) Data presented here are part of the ongoing ROADMAP-MM-CAT study and more data is expected to be released in the future.

3.1 Symptomatic Multiple myeloma population

A total of 144 eligible patients with NDMM were enrolled in the study from June 2014 to June 2017. No patients were lost on follow-up or excluded from analysis due to missing data. The demographics and clinical characteristics of the patients with symptomatic MM at the time of inclusion are summarized in Table 22. Median age was 66.0±11.6 (36-86) years and 53% of the population was male. Cardiovascular risk factors were present in 34% of patients. At inclusion all patients were naïve regarding any anti-myeloma treatment. Disease stage in the population was distributed as follows: 32% were ISS-I, 23% ISS-II and 45% ISS-III. Bone disease was present in 71% of patients and 27% had high risk cytogenetics. Proteasome inhibitor (PI) based therapy was given to 64% of patients, immunomodulatory drug (IMiD) based therapy in 32% and 4% received other regimens. Data and blood sample collection at the 3 month time point has been . After enrollment in the study and based on current thromboprophylaxis guidelines, current risk stratification and standard clinical care provided by the institution, 33% of patients did not receive any thromboprophylaxis, 51% received aspirin 100 mg o.d. and 16% received tinzaparin 4500 anti-Xa IU s.c. o.d. Median follow up time was 15.5 months (27 days – 35 months). During follow up 37 patients died.

Patients' clinical characteristics		Patients' biological data	(mean ± SD; range)
Age (years)	66.0±12.0 (36-86)		
Male/female	76/68 (53%/47%)		
BSA (m^2)	1.85±0.20 (1.46-2.50)	**β2-microglobulin (mg/dl)**	8.0±8.7 (0.06-48.5)
BMI (kg/m^2)	25.9±5.0 (17.2-44.8)	**M-peak (g/dl)**	2.9±2.3 (0-9)
ISS stage - n(%)		**U-peak (mg/24hours)**	356±846 (0-6667)
I	46 (32%)	**Bone marrow Infiltration (%)**	61.4±27.0 (0-100)
II	33 (23%)	**Total protein (g/dl)**	8.6±2.1 (5.1-14.3)
III	65 (45%)	**Creatinine (mg/dl)**	1.85±3.0 (0.47-28.0)
MM type - n(%)		**Urea (mg/dl)**	58.1±42.0 (5-276)
IgA	37 (26%)	**GFR (ml/min)**	73.0±43.0 (4.2-230.0)
IgG	80(55.5%)	**LDH (U/L)**	196±96 (70-789)
κLC	18(12.5%)	**ALT (U/L)**	24.3±21.0 (6-162)
λLC	9(6.0%)	**AST (U/L)**	24.1±24.0 (6-178)
Anti-myeloma treatment - n(%)		**Albumin (g/dl)**	3.8±0.7 (2.1-6.8)
PI-based	92 (64%)	**Calcium (mg/dl)**	9.7±1.1 (6.7-13.4)
IMiD-based	46 (32%)	**Hb (g/dl)**	10.5±2.0 (7.0-17.5)
Other	6 (4%)	**White blood cell count ($x10^6/\mu l$)**	6.8±3.0 (0.48-18.8)
ECOG performance status - n(%)		**Neutrophils ($x10^6/\mu l$)**	4.3±2.3 (0.2-12.6)
		Platelets ($x10^3/\mu l$)	256±125 (26-879)
0	57 (44.7)		
1	59(41)	**Thromboprophylaxis after**	
2	23(16)	**enrollment in the study**	N (%)
3	4 (2.8)		
4	1(0.7)	None	47 (33)
Dialysis at diagnosis - n(%)	14 (10%)	Aspirin	74 (51.0)
Bone disease present - n(%)	102 (71%)	LMWH (tinzaparin)	23 (16)
High risk cytogenetics- n(%)	27 (19%)		
Comorbidities and VTE risk factors non related with the cancer - n(%)			
Active pulmonary disease	13 (9%)		
CV risk factors	110 (76.4%)		
EPO use	50 (35%)		
GFR<30ml/min	22(15%)		

Table 22 Baseline demographic, clinical and biological characteristics of multiple myeloma patients. CV: cardiovascular, EPO: erythropoetin, ISS: International Staging system per ISS, VTE: ,venous thromboembolism; LMWH: low molecular weight heparin; PI: proteasome inhibitor based, IMiD: immunomodulatory drug based, MM: multiple myeloma, BMI: body mass index; BSA: body surface area; M-peak: serum monoclonal protein, U-peak: urine monoclonal protein, Hb: hemoglobin; LDH: lactate dehydrogenase; GFR: glomerular filtration rate; ALT: alanine aminotransferase; AST: aspartate aminotransferase; ECOG: Eastern Cooperative Oncology Group.

Eight patients with a new diagnosis of SMM were enrolled and had sample and data analysis completed up to date. Out of eighty SMM patients, 47 were male (46%), median age at diagnosis was 68.5 years, median BMI was 27kg/m^2, ECOG performance status was 0 in 90% and 58% had at least one cardiovascular risk factor. Median percentage of bone marrow infiltration was 20% (range 10-60%), 73% were IgG SMM patients, 21% were IgA SMM patients and 6% were light chain SMM. Mean Mpeak levels were 1.38 g/dl and Upeak 93.67 mg/24hours. During the follow up 4 out of 80 patients (5%) with SMM progressed to symptomatic disease. Median age of the 54 MGUS patients was 66.5 years of age, 33% only were male and median BMI was 1.65kg/m^2. ECOG performance status was 0 in 96% of MGUS patients and 54% had at least one cardiovascular risk factor. Median percentage of bone marrow infiltration was 6%, 70% were IgG MGUS, 8% were IgG MGUS, 15% were IgM MGUS and 7% were light chain MGUS. None of the MGUS patients progressed to symptomatic MM during follow up.

3.2 Follow-up and VTE

The overall rate of symptomatic VTE for the 144 MM patients during follow up (was 10.4% (n=15 out of 144 patients). The 1-year follow up for VTE occurrence was completed for all 144 patients. Nine out of 15 events (60%) occurred within 3 months from treatment initiation. Six of these patients did not receive any thromboprophylaxis; 6 patients were on aspirin at the time of the event and 3 were on LMWH. Out of the patients that received IMiD-based therapy 74% were on aspirin, 14% were on LMWH and 12% received no thromboprophylaxis. Out of the patients that received PI based treatment 54% received no thromboprophylaxis, 36% were on aspirin and 10% received LMWH. The rate of VTE did

notdiffer significantly between patients who received thromboprophylaxis and those who did not. The rate of VTE was not significantly different between patients who received IMiD-based (11.4%) treatment and patients on other therapy (10%). Almost half of the events were distal DVT (46.7%) and 13.3% were pulmonary embolism. Analytical data on patients with VTE are shown in Table 23. No events were reported for the SMM and MGUS groups.

3.3 Clinical and disease predictors for VTE

In a univariate analysis, among predictors related with patient's characteristics and underlying diseases, pulmonary disease (see definition in supplement) was significantly associated with the occurrence of symptomatic VTE (OR 4.85, 95% CI: 1.28 – 18.33; p = 0.020). Age older than 75 years, presence of cardiovascular risk factors or disease, sex, diabetes, BMI and performance status, cardiovascular disease, recent hospitalization, hyperlipidemia and hypertension were not associated with VTE occurrence.
Among disease specific characteristics the amount of monoclonal protein (*M-peak, in gr/dl)* was inversely associated with symptomatic VTE (OR = 0.72, 95% CI: 0.53 – 0.97; p=0.032) . On the other hand, the type of heavy chain, light chain, serum immunoglobulin levels, β2-microglobulin, Urine M-peak, ISS stage, BM infiltration percentage, presence of lytic bone lesions, abnormal WBCT and MRI pattern of infiltration were not associated with VTE.

3.4 Coagulation profile at diagnosis of multiple myeloma prior to treatment initiation

At inclusion, patients showed significantly increased levels of TFa, FVIIa, D-Dimers and FM and significantly shorter Procoag-PPL® as compared to the group of healthy individuals. Levels of P-selectin and TM were significantly lower in patients as compared to healthy individuals. The levels of heparanase were not significantly different in the group of patients as compared to the healthy individuals. Overall thrombin generation was attenuated in patients compared to healthy individuals. Lag-time and ttPeak were significantly increased and Peak, MRI and ETP were significantly lower as compared to the group of healthy individuals (Figure 3, Table 23). The TFPI and TM were positively correlated with chronometric parameters of thrombogram and negatively correlated with ETP and Peak.

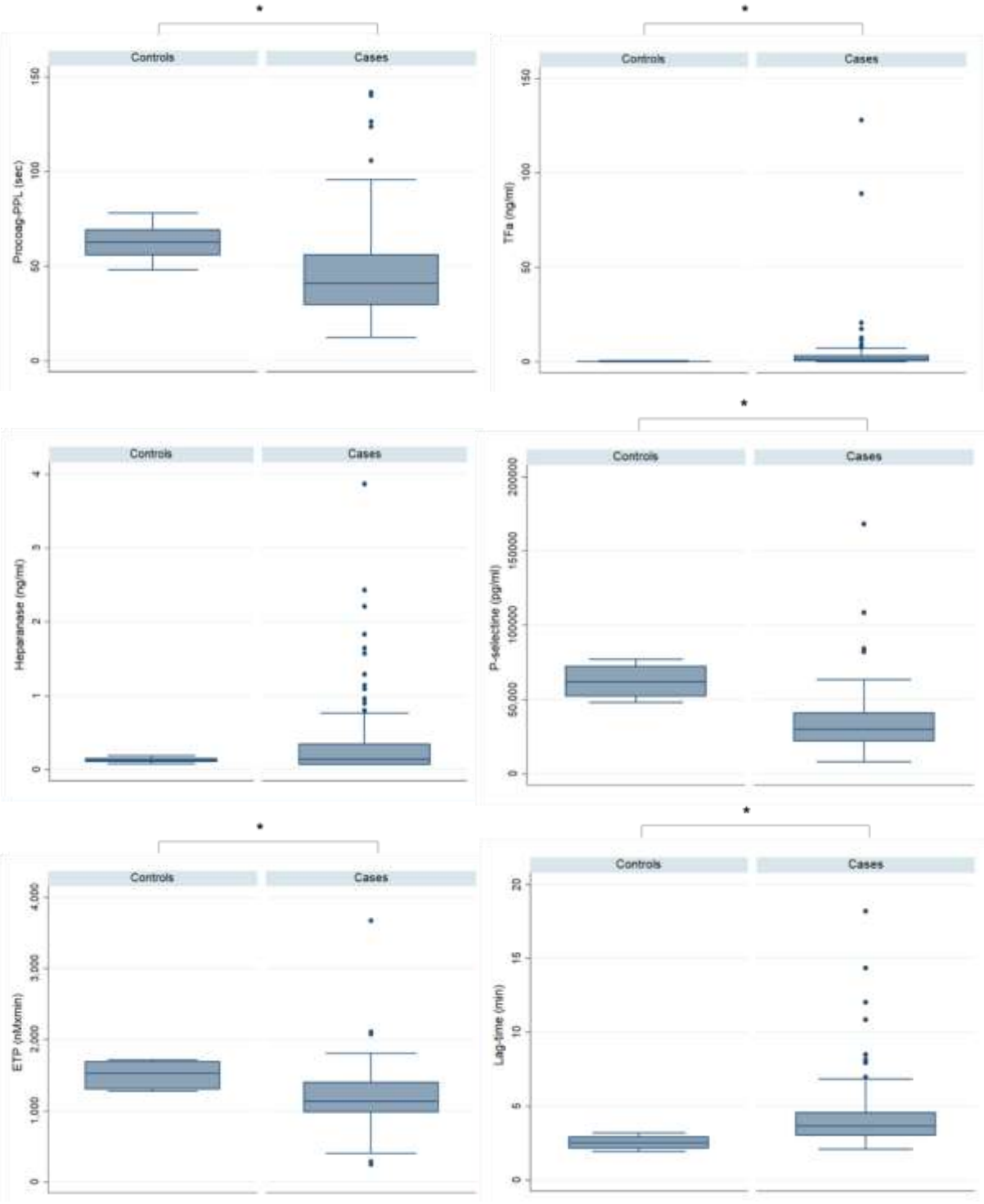
TFPI was also positively correlated with PPL-ct and TM. Results are presented in Table 24 and should be deemed explorative in view of multiple comparisons tested simultaneously.

Patients' identification #	sex	age	Localization	Time of event from diagnosis (days)	Disease status at follow up	Thrombo-prophylaxis	Anti-myeloma treatment
1A	M	50	IJV thrombosis post CVC insertion	150	PR	no	ASCT
2A	F	46	IJV thrombosis post CVC insertion	90	VGPR	no	ASCT
3A	M	40	Superficial UL vein thrombosis	90	PR	Aspirin	RAD
4A	F	76	Superficial LL vein thrombosis	60	PR	no	VMP
5A	M	78	distal DVT	45	PR	LMWH	CTD
6A	M	81	distal DVT	45	PR	Aspirin	VMP
7A	M	68	distal DVT	15	PR	no	VCD
8A	M	55	PE	180	PD	Aspirin	RD
9A	F	88	Mesenteric vein thrombosis	90	SD	LMWH	CTD
10A	M	62	distal DVT	360	VGPR	Aspirin	RD
11A	F	73	distal DVT	270	PR	LMWH (prior to event)	RD
12A	M	71	distal DVT	330	PR	aspirin	RD
13A	M	43	IJV thrombosis – CVC insertion	135	PR	no	ASCT
14A	F	81	distal DVT	30	SD	aspirin	RD
15A	M	59	PE	10	non evaluable	none	none

Table 23. Venous thromboembolism events among study population. M: male, F: female, DVT: deep vein thrombosis of the lower limb; CVC: central venous catheter insertion; IJV: internal jugular vein; LL: lower limb; UL: upper limb, PR: partial response; VGPR: very good partial response; PD: progressive disease; SD: stable disease; ASCT: autologous stem cell transplant; RAD: revlimid, adriamycin and dexamethasone; VMP: velcade, melphalan and prednisone; CTD: cyclophosphamide, thalidomide and dexamethasone; VCD: velcade, cyclophosphamide and dexamethasone; RD: revlimid and dexamethasone.

	Normal reference range	Healthy subjects (n=30)	MM (n=144)	p
Cellular derived hypercoagulability				
Procoag-PPL (sec.)	42 – 85	62.8±8.6	45.6±.22.6	<0.0001
TFa (ng/ml)	0.02 – 0.45	0.26±0.13	3.97±13.10	<0.0001
Heparanase (ng/ml)	0.08 – 0.16	0.13±0.03	0.34±0.52	0.476
TM (%)	70 - 120	90±18	39.25±68.1	<0.005
P-selectin (µg/ml)	82 - 42	62.66±103.91	38.12±31.78	<0.0001
TFPI (ng/ml)	15 - 26	18 ± 4 ng/ml	31±18.5	0.02
Blood coagulation factors and natural inhibitors				
FVIIa (U/ml)	73 – 29	50.9±10.6	74.1±147.6	0.022
FV (%)	70 - 120	90±12	78±11	0.23
AT (%)	70 – 120	92±12.0	95.4±17.7	<0.005
In vivo fibrin formation/lysis				
D-Dimers (µg/ml)	<0.50	0.31±0.08	1.80±3.41	<0.0001
FM (µg/ml)	0.5 – 5.50	2.5 ± 0.5	14.29±31.8	<0.0001
Thrombogram parameters				
Lag-time (min)	2.1 – 3.8	2.53±0.43	4.20±2.16	<0.0001
ttPeak (min)	4.0 – 6.6	5.28±0.73	7.33±2.76	<0.0001
Peak (nM)	222 – 330	287.8±35.7	214.4±80.1	<0.0001
MRI (nM/min)	60 – 120	109.9±24.5	80.2±45.7	<0.0001
ETP (nMxmin)	1600 - 1178	1496.8±191.4	1181.8±398	<0.0001

Table 23: Profile of hypercoagulability in patients at diagnosis of MM prior to treatment initiation. Procoag-PPL: procoagulant phospholipid dependent clotting time; TFa: tissue factor activity; TM: thrombomodulin activity; TFPI: tissue factor pathway inhibitor; FVIIa: activated factor VII; FV: factor V, ATIII: antithrombin; FM: fibrin monomers; ttPeak: time to peak of thrombin); MRI: mean rate index of thrombin generation; ETP: endogenous thrombin potential; p-values derived from Mann-Whitney-Wilcoxon test for independent samples (comparison of patients versus healthy individuals).


*
Controls
Cases
Procoag-PPL (sec)
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Cases
TFa (ng/ml)
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Heparanase (ng/ml)
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1
2
3
4
*
Controls
Cases
P-selectine (pg/ml)
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50.000
100000
150000
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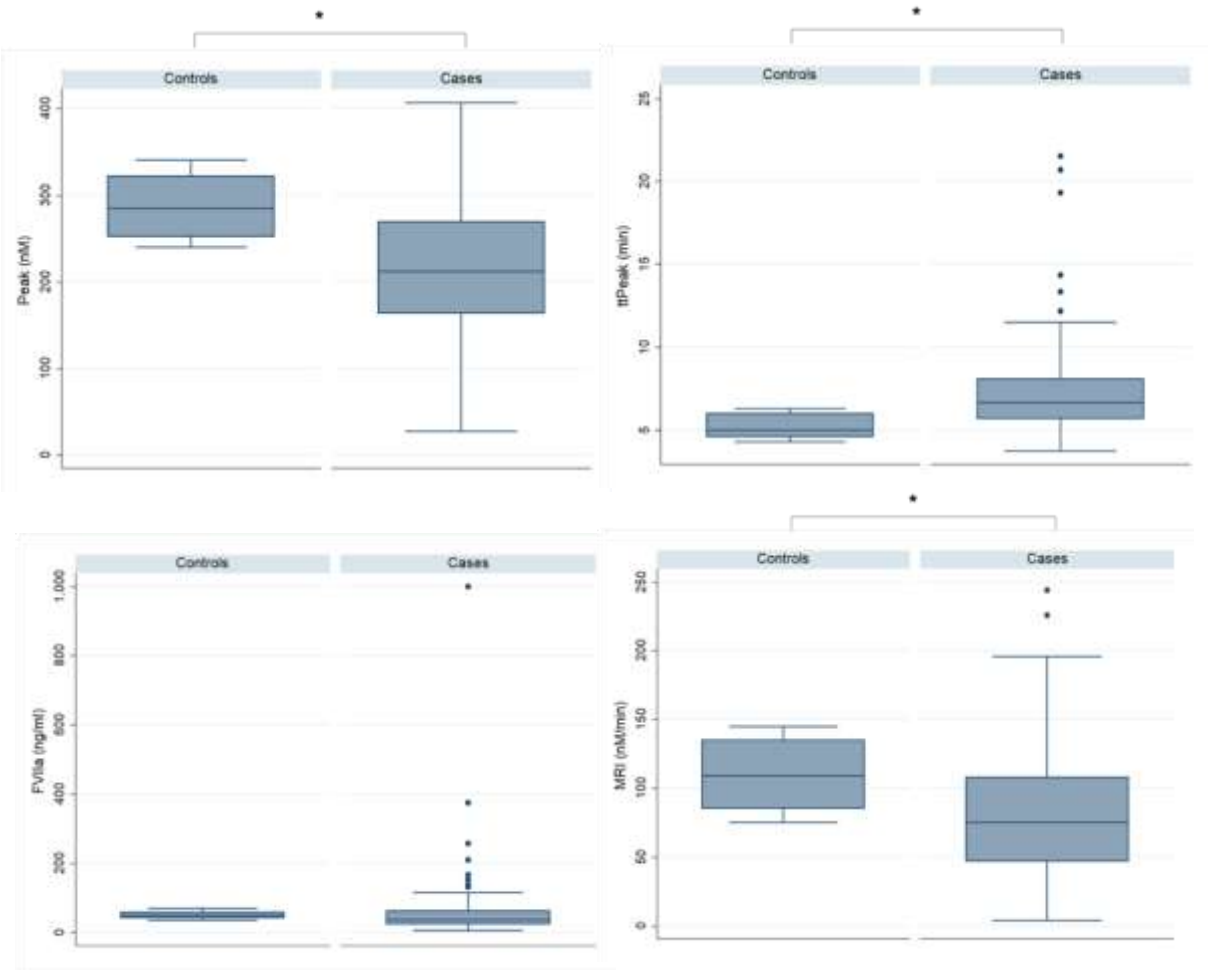

Figure 3: Boxplots showing the biomarkers in MM patients versus controls. PPL-ct (procoagulant phospholipid dependent clotting time); TFa (tissue factor activity); FVIIIa (activity of factor VIII); D-Di (D-dimers); ETP (the endogenous thrombin potential); MRI (mean rate index of thrombin generation); ttPeak (time to reach the peak concentration of thrombin). * = p<0.0001

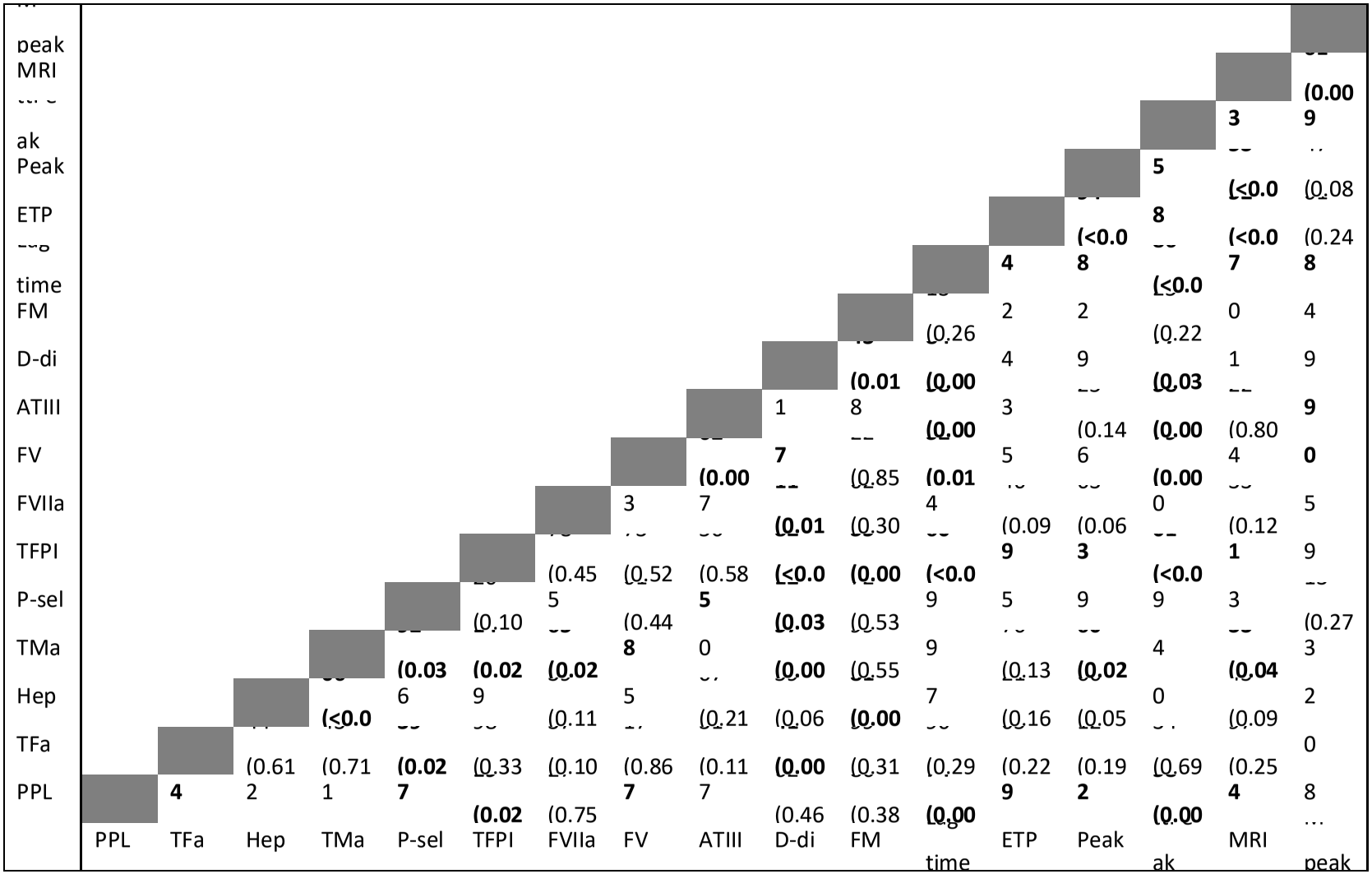
PPL
TFa
Hep
TMa
P-sel
TFPI
FVIIa
FV
ATIII
D-di
FM
time
ETP
Peak
ak
MRI
peak

Table 24. Spearman's rank correlation coefficient (p-values in brackets) showing the intercorrelations between hypercoagulability biomarkers and M-peak assessed in patients before treatment administration. Bold cells denote intercorrelations with p<0.05.

3.5 Coagulation profile in patients with SMM and MGUS:

Biomarker analysis at the time of study enrollment has also been completed for a total of 80 patients with SMM and 54 patients with MGUS. Coagulation biomarkers in SMM patients were compared to healthy subjects. As seen in table 25 procoag-PPL time was shorter, the levels of TFa, heparanase, TM, FM and AT were significantly higher in patients with SMM versus healthy subjects, P-selectin levels were significantly lower and levels of FVIIa, FV and D-dimer were not significantly different between the two groups. All thrombogram parameters differed significantly between SMM patients and healthy subjects; lagtime was longer, ttPeak shorter, peak concentration levels higher and MRI and ETP smaller in SMM patients versus healthy subjects.

Biomarkers in MGUS patients were compared against healthy subjects as well. (table 26) Procoagulant PPL-ct was shorter in MGUS patients, levels of FTa, heparanase, TM, AT, D-dimer and FM were significantly higher and P-selectin levels significantly lower in MGUS patients. Lagtime is increased, ttPeak longer, peak concentration, Peak concentration MRI and ETP lower.

Biomarkers were also compared directly between SMM and MM patients and MGUS and MM patients. In table 27 it can be seen that between SMM and MM patients, only P-selectin, D-dimer and FM levels differed significantly and were higher in patients with MM; P-selectin levels 38.12±31.78 μg/ml in MM patients vs 32.23 ± 35.10 μg/ml in SMM patients, *p=0.014*, D-dimer levels 1.80±3.41 μg/ml in MM patients vs 0.48±0.45 μg/ml in SMM patients, *p<0.0001* and FM levels 14.29±31.8 μg/ml in MM patients vs 11.45±18.88 μg/ml in SMM patients, *p=0.018*. In the equivalent comparison between MGUS patients and MM patients D-dimer levels were higher in MM patients, Peak thrombin centration and MRI of the thrombogram significantly lower in MGUS patients. (table 27).

Parameters	SMM (n=80)	Healthy subjects (n=30)	p§
	Cellular derived hypercoagulability		
Procoag-PPL (sec)	48.18±23.03	62.8±8.64	**0.0005**
TFa (ng/ml)	3.73±13.45	0.26±0.13	**<0.0001**
Heparanase (ng/ml)	0.44±0.47	0.13±0.03	**<0.0001**
TM (%)	129.47±89.58	90±18	
P-Selectin (pg/ml)	32.23 ± 35.10	62.66 ±103.91	**<0.0001**
	Blood coagulation factors and natural inhibitors		
F.VIIa (U/ml)	84.21±25.7	50.9±10.63	0.262
FV (%)	84.21±25.7	90±12	0.28
AT (%)	97.26±15.94	92±12.0	**<0.005**
	In vivo fibrin formation/lysis		
D-Di (µg/ml)	0.48±0.45	0.31±0.08	0.178
FM (µg/ml)	11.45±18.88	2.5 ± 0.5	**<0.0001**
	Thrombogram parameters		
Lagtime (min)	3.4±0.67	2.53±0.43	**<0.0001**
ttPeak (min)	6.51±1.18	287.8±35.7	**<0.0001**
Peak (nM)	232.41±68.95	5.28±0.73	**<0.0001**
MRI	83.82±40.54	109.9±24.46	**0.0001**
ETP (nM/min)	1243.02±286.71	1496.83±191.4	**<0.0001**

Table 25: Differences in coagulation markers between SMM patients and healthy subjects.
Procoag-PPL: procoagulant phospholipid dependent clotting time; TFa: tissue factor activity; TM: thrombomodulin activity; TFPI: tissue factor pathway inhibitor; FVIIa: activated factor VII; FV: factor V, ATIII: antithrombin; FM: fibrin monomers; ttPeak: time to peak of thrombin); MRI: mean rate index of thrombin generation; ETP: endogenous thrombin potential; p-values derived from Mann-Whitney-Wilcoxon test for independent samples.

Parameters	MGUS (n=54)	Healthy subjects (n=30)	p
Cellular derived hypercoagulability			
Procoag -PPL (sec)	44.06±13.99	62.8±8.64	**0.0001**
FTa (ng/ml)	3.62±4.6	0.26±0.13	**<0.0001**
Heparanase (ng/ml)	0.39±0.27	0.13±0.03	**<0.0001**
TM (%)	162.85±56.95	90±18	<0.0001
P-Selectin (pg/ml)	38.79 ±28.10	62.66 ±103.91	**<0.0001**
Blood coagulation factors and natural inhibitors			
F.VIIa (U/ml)	123.59±259.42	50.9±10.63	0.743
FV (%)	73.08±22.11	90±12	0.081
ATIII (%)	100.31±11.61	92±12.0	**<0.005**
In vivo fibrin formation/lysis			
D-Di (µg/ml)	0.99±1.17	0.31±0.08	**0.004**
FM (µg/ml)	12.87±18.71	2.5 ± 0.5	**<0.0001**
Thrombogram parameters			
Lagtime (min)	3.6±1.18	2.53±0.43	**<0.0002**
ttPeak (min)	7.14±1.93	5.28±0.73	**<0.0001**
Peak (nM)	188.69±77.64	287.8±35.7	**<0.0001**
MRI	63.71±46.27	109.9±24.5	**<0.0001**
ETP (nM/min)	1101.21±290.55	1496.8±191.4	**<0.0001**

Table 26: Differences in coagulation markers between MGUS patients and healthy subjects. Procoag-PPL: procoagulant phospholipid dependent clotting time; TFa: tissue factor activity; TM: thrombomodulin activity; TFPI: tissue factor pathway inhibitor; FVIIa: activated factor VII; FV: factor V, ATIII: antithrombin; FM: fibrin monomers; ttPeak: time to peak of thrombin); MRI: mean rate index of thrombin generation; ETP: endogenous thrombin potential; p-values derived from Mann-Whitney-Wilcoxon test for independent samples.

	SMM (n=53)	MM (n=144)	p
Procoag-PPL (sec.)	48.18±23.03	45.6±.22.6	0.161
TFa (ng/ml)	3.73±13.45	3.97±13.10	0.586
Heparanase (ng/ml)	0.44±0.47	0.34±0.52	0.318
TM (%)	129.47±89.58	39.25±68.1	0.914
P-selectin (µg/ml)	32.23 ± 35.10	38.12±31.78	**0.014**
FVIIa (U/ml)	108.26±229.95	74.1±147.6	0.799
FV (%)	84.21±25.7	78±11	0.638
AT (%)	97.26±15.94	95.4±17.7	0.482
D-Dimers (µg/ml)	0.48±0.45	1.80±3.41	**<0.0001**
FM (µg/ml)	11.45±18.88	14.29±31.8	**0.018**
Lag-time (min)	3.4±0.67	4.20±2.16	0.059
ttPeak (min)	6.51±1.18	7.33±2.76	0.588
Peak (nM)	232.41±68.95	214.4±80.1	0.408
MRI (nM/min)	83.82±40.54	80.2±45.7	0.97
ETP (nMxmin)	1243.02±286.71	1181.8±398	0.157

Table 27: comparison of biomarkers between SMM and MM patients. Procoag-PPL: procoagulant phospholipid dependent clotting time; TFa: tissue factor activity; TM: thrombomodulin activity; TFPI: tissue factor pathway inhibitor; FVIIa: activated factor VII; FV: factor V, ATIII: antithrombin; FM: fibrin monomers; ttPeak: time to peak of thrombin); MRI: mean rate index of thrombin generation; ETP: endogenous thrombin potential. §p-values derived from Mann-Whitney-Wilcoxon test for independent samples.

Along the MGUS-SMM and MM continuum, MM patients presented with significantly higher D-dimer levels, FM levels and longer lagtime (table 28).

Parameters	MGUS (n=54)	SMM (n=80)	MM (n=144)	Spearman's rho	p
Cellular derived hypercoagulability					
PPL (sec)	44.06±13.99	48.18±23.03	45.6±.22.6	-0.073	0.382
FTa (ng/ml)	3.62±4.6	3.73±13.45	3.97±13.10	-0.050	0.554
Heparanase (ng/ml)	0.39±0.27	0.44±0.47	0.34±0.52	-0.139	0.099
TM (%)	162.85±56.95	129.47±89.58	39.25±68.1	-0.143	0.251
P-Selectin (pg/ml)	38.79 ±28.10	32.23 ± 35.10	38.12±31.78	+0.038	0.650
Blood coagulation and natural inhibitors					
F.VIIa (U/ml)	123.59±259.42	108.26±229.95	74.1±147.6	+0.012	0.887
FV (%)	73.08±22.11	84.21±25.7	78±11	+0.170	0.153
ATIII (%)	100.31±11.61	97.26±15.94	95.4±17.7	-0.116	0.166
In vivo fibrin formation/lysis					
D-Di (µg/ml)	0.99±1.17	0.48±0.45	1.80±3.41	+0.436	**<0.0001**
FM (µg/ml)	12.87±18.71	11.45±18.88	14.29±31.8	+0.179	**0.031**
THrombogram parameters					
Lagtime (min)	3.6±1.18	3.4±0.67	4.20±2.16	+0.185	**0.027**
ttPeak (min)	7.14±1.93	6.51±1.18	7.33±2.76	+0.014	0.865
Peak (nM)	188.69±77.64	232.41±68.95	214.4±80.1	+0.133	0.114
MRI	63.71±46.27	83.82±40.54	80.2±45.7	+0.184	0.130
ETP (nM/min)	1101.21±290.55	1243.02±286.71	1181.8±398	+0.052	0.534

Table 28: Differences in coagulation markers along the MGUS-SMM and MM continuum: Procoag-PPL: procoagulant phospholipid dependent clotting time; TFa: tissue factor activity; TM: thrombomodulin activity; TFPI: tissue factor pathway inhibitor; FVIIa: activated factor VII; FV: factor V, ATIII: antithrombin; FM: fibrin monomers; ttPeak: time to peak of thrombin); MRI: mean rate index of thrombin generation; ETP: endogenous thrombin potential; p-values derived from Mann-Whitney-Wilcoxon test for independent samples (comparison of patients versus healthy individuals).

3.6 Coagulation profile at 3 month follow up:

Blood sample collection, coagulation biomarker measurements and data analysis has been completed at the first time point of the study (3 months post treatment initiation) in a total of 130 patient with MM. As seen in table 29 after 3 months of anti-myeloma treatment TF levels decreased significantly (0.92±1.38 ng/ml at T1 versus 4.22±14.70 ng/ml at T0 *p=0.019*), TFPI levels decreased (30.33±11.9 ng/ml at T1 vs 34.8±15.9 ng/ml at T0, *p=0.04*), FVII levels

	Normal reference range	**MM (n= 130) at T0**	**MM (n=130) at T1**	**p**
Cellular derived hypercoagulability				
Procoag-PPL (sec.)	42 – 85	47.4±.24.8	43.6±12.6	0.14
TFa (ng/ml)	0.02 – 0.45	4.22±14.70	0.92±1.38	**0.019**
Heparanase (ng/ml)	0.08 – 0.16	0.35±0.53	0.42±0.75	0.49
TM (%)	70 - 120	49.35±48.1	44.4 ± 48.7	0.63
P-selectin (µg/ml)	82 - 42	37.25±27.15	29.20±15.3	0.06
TFPI (ng/ml)	15 - 26	34.8±15.9	30.33±11.9	**0.04**
Blood coagulation factors and natural inhibitors				
FVIIa (U/ml)	73 – 29	73.9±161.0	41.2±25.2	**0.034**
FV (%)	70 - 120	87.8±35.4	104.5±29.1	**<0.001**
AT (%)	70 – 120	94.4±18.1	98.6±13.5	0.05
In vivo fibrin formation/lysis				
D-Dimers (µg/ml)	<0.50	1.40±1.60	0.91±1.44	**0.014**
FM (µg/ml)	0.5 – 5.50	18.2±35.1	13.8±29.6	0.42
Thrombogram parameters				
Lag-time (min)	2.1 – 3.8	4.32±2.30	4.1±2.04	0.46
ttPeak (min)	4.0 – 6.6	7.47±2.90	8.02±2.83	0.16
Peak (nM)	222 – 330	210.7±78.0	171.3±80.9	**<0.001**
MRI (nM/min)	60 – 120	77.9±43.4	53.8±37.4	**<0.0001**
ETP (nMxmin)	1600 - 1178	1185.8±426	1033.4±337	**<0.01**

Table 29: Comparison of biomarker levels between baseline timepoint (T0) and second timepoint (T1) at 3 months in 130 patients with Multiple myeloma. Procoag-PPL: procoagulant phospholipid dependent clotting time; TFa: tissue factor activity; TM: thrombomodulin activity; TFPI: tissue factor pathway inhibitor; FVIIa: activated factor VII; FV: factor V, ATIII: antithrombin; FM: fibrin monomers;

ttPeak: time to peak of thrombin); MRI: mean rate index of thrombin generation; ETP: endogenous thrombin potential;

decreased (41.2±25.2 U/ml at T1 versus 74.1±147.6 U/ml at T0, *p=0.034),* FV levels increased (104.5±29.1 % at T1 versus 87.8±35.4 % at T0, *p<0.001*), D-dimer levels decreased (0.91±1.44 μg/ml at T1 versus1.40±1.60 μg/ml at T0 *p=0.14*), Peak thrombogram concentration (171.3±80.9 nM at T1 versus 210.7±78.0 nM at T0 *p<0.001*) and MRI (53.8±37.4 nM/min at T1 versus 77.9±43.4 nM/min at T0 *p<0.0001*) decreased. Sub-analysis based on the type of treatment patients received (IMiD-based versus PI-based) did not demonstrate any significant differences in the pattern of biomarker change at 3 months.

3.7 Biomarkers as Predictors of VTE

ROC analysis defined cut-offs for biomarkers and univariate analysis was performed. The analysis showed that patients with Procoag-PPL® ≥47 had a 3.49 times higher risk of VTE compared to patients with Procoag-PPL® <47 sec (OR=3.49, 95% CI: 1.13-10.82, p=0.030). In addition, patients with ETP ≥ 1087 nMxmin versus patients with ETP < 1087 nMxmin had significantly lower risk of VTE (OR =0.25 95% CI 0.07-0.83, p=0.024). Finally, patients with TFPI ≥39 versus patients with TFPI <39 had a 7.75 higher risk of VTE (OR=7.74 95% CI 1.51-39.70, p=0.014). (table 30)

	Compared categories	OR (95% CI)	p
Cellular derived hypercoagulability			
Procoag-PPL (sec)	≥47.0 vs. <47.0	**3.49 (1.13-0.82)**	**0.030**
TFa (ng/ml)	≥0.03 vs. <0.03	0.49 (0.09-2.50)	0.389
Heparanase (ng/ml)	≥0.68 vs. <0.68	Not estimable due to zero events in the upper category	0.215 [F]
TMa (%)	≥42.0 vs. <42.0	4.93 (0.97-24.99)	0.054
P-selectin (pg/ml)	≥46700 vs. <46700	2.69 (0.71-10.26)	0.147
TFPI (ng/ml)	≥39.0 vs. <39.0	**7.75 (1.51-39.70)**	**0.014**
Blood coagulation factors and natural inhibitors			
FVIIa (ng/ml)	≥56.8 vs. <56.8	0.34 (0.07-1.59)	0.172
FV (%)	≥103 vs. <103	0.15 (0.02-1.18)	0.071
ATIII (%)	≥87 vs. <87	2.33 (0.50-10.84)	0.282
In vivo thrombin generation			
D-Dimers (µg/ml)	≥2.1 vs. <2.1	2.52 (0.82-7.69)	0.105
FM (µg/ml)	≥8.4 vs. <8.4	2.07 (0.61-6.95)	0.241
Thrombogram parameters			
Lag-time (min)	≥6.5 vs. <6.5	Not estimable due to zero events in the upper category	0.612 [F]
ETP (Mxmin)	≥1087 vs. <1087	**0.25 (0.07-0.83)**	**0.024**
Peak (nM)	≥253.0 vs. <253.0	1.50 (0.49-4.61)	0.479
ttPeak (min)	≥10 vs. <10	Not estimable due to zero events in the upper category	0.364 [F]
MRI (nM/min)	≥121 vs. <121	1.40 (0.36-5.49)	0.625

Table 30: Univariate logistic regression analysis evaluating associations between the examined biomarkers and VTE. The cut-off levels were set on the basis of the respective ROC curves. PPL-ct (procoagulant phospholipid dependent clotting time); TFa (tissue factor activity); TM: thrombomodulin activity; TFPI: tissue factor pathway inhibitor; FVIIa (activity of factor VII); FV (factor V); ATIII (anti-thrombin); FM (fibrin monomer); ETP (the endogenous thrombin potential); Peak (the peak concentration of thrombin); ttPeak (time to reach the peak concentration of thrombin); MRI (mean rate index of thrombin generation); p-values derived from Mann-Whitney-Wilcoxon test for independent samples

3.8 Multivariate logistic regression and model equation:

The type of thromboprophylaxis (none; aspirin; LMWH) was not associated with VTE risk (p=0.535, Fisher's exact test). Therefore, thromboprophylaxis was not entered into the

multivariate logistic regression analysis. Following systematic testing by univariate analysis of all biomarkers we identified the significant predictors for use in a multivariate model. Multivariate logistic regression analysis demonstrated that ETP <1087 nMxmin versus ≥1087 nMxmin (OR=4.04, 95% CI 1.18-13.84, p=0.026) and Procoag-PPL® ≥47 versus <47 sec (OR=3.01, 95% CI 0.93-9.78, p=0.066), were independently associated with VTE occurrence.

This multivariate logistic regression model corresponded to the following equation:
log(odds for VTE) = -3.51 + (1.40*ETP_binary)+ (1.10*Procoag-PPL®_binary)

Based on the equation above, a score was formulated where the dependent variable is the log(odds for VTE) and the binary predictors: 1 for Procoag-PPL® ≥47, and 1 for ETP <1087 nMxmin), or 0 for Procoag-PPL® <47 sec, and ETP ≥1087 nMxmin respectively. The AUC of the ROC analysis was 0.73. Patients were stratified at high or intermediate/low risk group; the optimal cut-off level in the aforementioned score was equal to -2.11. The rate of VTE was 5% in the intermediate/low risk group and 17.5% in the high risk group. The rate of VTE was not significantly associated with the type of thromboprophylaxis in either the intermediate/low risk group (p=0.62, Fisher's exact test) or the high risk group (p=0.588, Fisher's exact test). The sensitivity and the specificity of the score was 71.4% and 61.8%, respectively. According to the Hosmer-Lemeshow test, a value of p = 0.858 showed that the model was well calibrated.

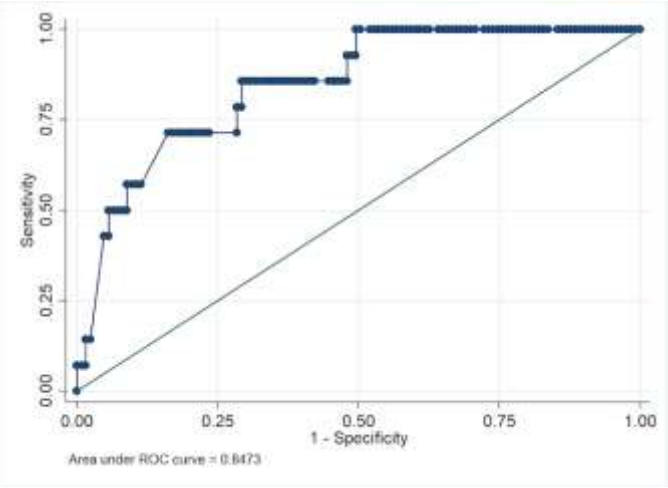

Figure 4: Area under the curve (AUC) of the ROC analysis performed.

4 DISCUSSION:

The prospective observational ROADMAP-MM-CAT aimed to explore the complex coagulation profile of newly diagnosed, chemotherapy naïve patients with symptomatic MM. The ultimate goal of this ongoing study is to identify clinically relevant biomarkers for use in VTE risk stratification and the development of a RAM. As part of this ongoing study we have also explored the coagulation profile of patients with SMM and MGUS, the precursor states of MM.

The results presented provide biological evidence linking cell derived hypercoagulability with MM disease and VTE risk. A new score was derived based on biomarkers of hypercoagulability which accurately stratified patients as high and intermediate/low risk for VTE. The rate of symptomatic VTE was 10.4% among MM patients, it was highest during the first 4 months following diagnosis and distal DVT was the most frequent localization, all of which agree with recent literature. (354, 355, 457) (338) Despite initial risk stratification and application of pharmacological thromboprophylaxis, VTE events occurred across all risk groups. The study design did not allow detection of potential differences in efficacy and safety of thromboprophylaxis with aspirin or LMWH in patients stratified as intermediate/low or high risk for VTE. The data therefore confirm that the residual rate of symptomatic VTE remains high and point out the need an alternative antithrombotic strategy in this population with optimization of the tools used to select patients eligible for thromboprophylaxis. (91, 445)

The study is prospective, and the clinical features of the derivation cohort respond to the principal generalizability criteria for risk assessment tools (239, 397, 467). Pre-entry VTE status was assessed with Doppler echography in a sensitive, cost-effective and patient friendly manner. VTE risk assessment was performed at baseline according to clinical practice. A large number of hypercoagulability biomarkers were assessed prior to treatment administration. Among them, procoagulant phospholipid-dependent clotting time (Procoag-PPL®), endogenous thrombin generation potential (assessed in PPP with the Calibrated

Automated Thrombogram-Thrombinoscope® assay using the 5 pM TF PPP-Reagent®) and the levels of TFPI were significant predictors of VTE risk. Multivariate analysis led to derivation of the new ROADMAP-CAT-MM score which combines Procoag-PPL® clotting time and ETP and accurately stratifies patients into high and intermediate/low risk for VTE. The sensitivity and the specificity of the new score is 71.4% and 61.8%, respectively and the AUC of the ROC analysis is 0.73.

Procoag-PPL® clotting time correlates with and reflects the concentration of procoagulant microparticles derived from platelets or other cells. (252) All patients had shorter Procoag-PPL® clotting time than the lower normal levels of this test indicating that platelet and/or endothelial cell activation is linked with MM disease. Following 3 months of treatment Procoag-PPL clotting time did not change significantly indicating that endothelial and/or platelet activation continues and that 3 months is an inadequate amount of time to shift the balance in the complex interactions between the monoclonal plasma cells and biomarkers of hypercoagulability. P-selectin levels (a marker of platelet activation) were also low in patients with MM compared with healthy subjects. This is thought to reflect a state of chronic platelet activation which has previously been described as "exhausted platelet syndrome" (468, 469). Sustained platelet activation could be part of the Myeloma disease process. At 3 months P-selectin levels did not change significantly possibly indicating the need to assess changes in coagulation biomarkers over longer periods of treatment exposure. Endothelial cell activation is reflected by the increased levels of TFa and TFPI in patients (15-fold and 1.7-fold respectively as compared to those in healthy individuals). TF is the initiator of coagulation whereas TFPI belongs to the natural anticoagulant system but both proteins are synthesized and released by activated endothelial cells (470, 471). Consequently, the increase of these biomarkers, along with the increase of FVIIa, indicates endothelial cell activation. Following 3 months of treatment levels of TFa, TFPI and FVIIa all decrease significantly. Given the more direct and clear-cut roles of these biomarkers in the coagulation pathways one could hypothesize that these return to normal early following response to anti-myeloma treatment.

Myeloma patients also show an unexpected attenuation of thrombin generation in plasma. Lag-time and ttPeak were significantly prolonged, whereas Peak, MRI and ETP were significantly lower as compared to healthy individuals. Following treatment there is a further decrease in MRI and Peak concentration of thrombin. This finding may seem a paradox but is in agreement with the data published by Legendre et al (397). A methodological approach is required for the interpretation of this finding. Thrombogram-Thrombinoscope® assay is performed with exogenously added optimal concentrations of TF (5 pM) and procoagulant phospholipids (4 μM). Thus, the sensitivity of the test to variations of plasma concentrations of TF or procoagulant phospholipids is limited. In our study under the methodological conditions used the test is sensitive to the variations of TFPI and/or TM levels in plasma. Correlation analysis showed that the attenuation of thrombin generation is related to increased plasma concentration of TFPI and TM. Three months of treatment seems to be an inadequate period of time to allow reversal of the observed thrombin generation attenuation. In patients with NDMM, thrombin generation attenuation should be interpreted as a reflection of endothelial cell activation rather than as an indicator of plasma hypercoagulability; i.e. an imbalance between clotting factors and natural coagulation inhibitors. The increased levels of fibrin monomers and D-Dimers found in NDMM patients' plasma points to an environment of increased plasma hypercoagulability which is in accordance with previous studies (467, 472). Three months post treatment, D-dimer levels decreased significantly but FM did not change to a significant extent.

Patients with SMM and MGUS had a similar but not identical coagulation profile to symptomatic MM patients. PPL-ct was significantly shorter than healthy controls in SMM and in MGUS patients in a similar manner to symptomatic MM patients. TFa, TM, AT, FM and heparanase levels were higher in SMM and MGUS patients compared to healthy subjects. FV and FVIII levels were not significantly different compared to healthy controls in either group. D-dimer levels were shown to be higher only in MGUS patients and not SMM patients which was not expected. SMM and MGUS patients also showed lower levels of P-selectin compared to healthy controls. The pattern of thrombin generation is altered in SMM patients but it is not as clearly attenuated as in the symptomatic MM population. Lagtime is longer and MRI and ETP smaller like in the symptomatic stage but Peak

concentration is higher and ttPeak shorter. In MGUS patients lagtime and ttPeak are longer compared to controls but ETP, peak concentration and MRI lower. Thrombin generation is therefore attenuated even in the precursor MGUS state indicating that endothelial activation might be a process that is initiated at the very early stages of the disease.

The ROADMAP-MM study also identified pulmonary disease, defined as the presence of active chronic inflammatory obstructive disease requiring treatment, as the most relevant clinical predictor of VTE in NDMM patients. Other clinical predictors established for CAT in solid tumors such as age, immobilization recent hospitalization etc, were not identified as significant for MM associated VTE. This probably reflects the differences in the mechanism of thromboembolic disease pathogenesis between MM and solid tumors. This finding will need to be validated in future larger cohorts to assess its significance.

Interestingly, the M-peak, a myeloma specific biomarker is inversely correlated with VTE risk. Previously Carr et al showed that higher IgG levels induced thin fiber formation (380) whereas Undas et al showed that fibrin clots in MM patients are denser compared to healthy controls. (371) Our findings introduce the M-peak among the clinically relevant predictors of VTE in NDMM patients. The mechanisms by which the levels of M-peak influence the risk of VTE, taking into consideration the contradictory results of the literature will be studied in a future study from our group.

We aimed to identify biomarkers of hypercoagulability as predictors for VTE risk. In patients with MM Procoag-PPL® clotting time ≥47s led to a 3.5-fold higher risk of VTE as compared to those with a Procoag-PPL® clotting time shorter than this cut-off. This is an unexpected finding. One hypothesis is that this finding lies within the same context as Pselectin decrease and is eventually a reflection of the status of platelet exhaustion. In addition to procoag-PPL clotting time, lower ETP and higher TFPI levels were associated with an increased VTE risk. Patients with ETP ≥ 1087 nMxmin versus patients with ETP < 1087 nMxmin had a lower VTE risk. In addition, patients with TFPI ≥39 ng/ml versus those with TFPI <39 ng/ml had a 7.75 higher VTE risk (OR=7.74 95% CI (1.51-39.70). These findings further support the hypothesized concept that TFPI levels increase in plasma and that

thrombin generation attenuation reflects endothelial cell activation rather than a real down-regulation of plasma hypercoagulable state.

The feasibility of the ROADMAP-CAT-MM score proposed for VTE risk could prove to be challenging with the current technological expertise availability. Both biomarkers, Procoag-PPL® and ETP are commercially available, easy to perform and do not require a specialized laboratory infrastructure. They are however not routinely performed in most hospital laboratories and would require additional training and equipment. Procoag-PPL® clotting time can be installed in any blood coagulation analyzer and is a commercially available, user-friendly, fully automated, quick and reproducible technique. (473) ETP measurement is performed in PPP with the TF 5pM PPP-Reagent® using the Calibrated Automated Thrombogram-Thrombinoscope® assay and is also an automated, standardized technique, available in the market worldwide. (474, 475). The new version of the Calibrated Automated Thrombogram-Thrombinoscope® analyzer recently presented by the manufacturer, will render this method accessible to hematological laboratories which are not highly specialized in blood coagulation exploration. A financial analysis needs to be performed to ensure that the benefits of the new score will not be restricted by the cost of the laboratory assessment.

The monocentric design of the present study did not allow the assessment of the potential influence of other therapeutic practices or supportive treatments on the predictive capacity of the studied biomarkers. It is interesting to note that the VTE rate did not differ between patients who received IMid-based treatment versus other treatments. Given that thromboprophylaxis was applied to all patients based on current guidelines it is hypothesized that the residual VTE risk reflects the limitation of the actual algorithm for VTE assessment and suboptimal thromboprophylaxis administration. The sample size was sufficient to provide adequate statistical power for the derivation of the new score but a larger cohort is required to perform internal validation of the model. It should also be noted that the findings of the study are restricted to the assessment of thrombin generation and Procoag-PPL clotting time using the assays and reagents employed in the study design, not other methods.

As part of this project we also aim to identify among the studied hypercoagulability biomarkers the ones that are most clinically relevant for inclusion in treatment resistance assessment tools. Crosstalk between myeloma plasma cells with platelets and endothelial cells enhances hypercoagulability and consequently biomarkers of hypercoagulability are potential candidates worth assessing for the role in predicting outcomes.[477],[478] Cancer cells induce activation of platelets, endothelial cells or blood coagulation mechanism, either directly via the expression of procoagulant molecules and the release of procoagulant microparticles or indirectly via enhancement of the inflammatory reaction. (449, 478-480) On the other hand, the activation of platelets and blood coagulation enhances proliferation and metastasis of cancer cells and offers a shield against the immunosurveillance mechanisms.[481]

The concept of biomarker incorporation into clinical RAMs in order to improve their accuracy in the stratification of VTE risk in ambulatory cancer patients has been tested previously. (476) The incorporation of P-selectin and D-dimers in the Vienna prediction score improved the sensitivity and specificity of the original Khorana score for chemotherapy related VTE risk in patients with solid tumors. (245) (467) (246) (238) Similarly the incorporation of the Procoag-PPL® and MRI of the Calibrated Automated Thrombogram-Thrombinoscope® improved the accuracy of the COMPASS–CAT RAM for CAT in patients with solid tumors. (259) (476) Two clinical scores have been recently developed and presented for patients with MM. The IMPEDE score includes the use of IMiDs, BMI, presence of Pathologic fracture pelvis/femur, use of EPO, administration of Dexamethasone/Doxorubicin, Ethnicity/Race, history of VTE, presence of Tunneled line/CVC 2 point and the use of anticoagulation (453) and the SAVED score combines 7 weighted similar clinical factors to the IMPEDE score. (454) Both scores were developed retrospectively. We have developed prospectively the ROADMAP-MM biomarker score which will be incorporated into a clinical RAM for more accurate stratification of NDMM patients and administration of thromboprophylaxis in those classified as high risk of VTE. The combined RAM and score should be taken into consideration in the design of phase III clinical trials that aim to assess the efficacy and safety of differ thromboprophylaxis agents.

The need for RCT's designed to compare modes of thromboprophylaxis in MM patients based on clear cut risk stratification criteria is ever increasing. Robust clinical data are needed for optimum management of this significant complication in MM patients. The use of anticoagulation either for prophylaxis or treatment is rather complicated in patients with cancer and particularly with hematological malignancies. A number of issues need to be taken into consideration when deciding to initiate anticoagulants in MM patients to ensure not only efficacy but also safety. Thrombocytopenia is a common disease complication either due to the disease itself and bone marrow infiltration or secondary to anti-myeloma treatment. Careful monitoring is required to ensure anticoagulation is only applied when the platelet count is within safe levels. Most clinicians opt for anticoagulation when the platelet count is above 50.000. Renal impairment is also a very common complication of the disease which often restricts the choice of the anticoagulant agent. Finally careful review of the anti-myeloma agents and supportive treatment and possible interactions with DOACs/NOACs is required before initiation of thromboprophylaxis. Data from RCTs will hopefully answer these questions specifically for the MM patient as there are multiple unique parameters that need to be taken into consideration.

The ROADMAP-MM-CAT study is ongoing. Patient enrollment continues and as per protocol patients are being followed up including blood samples collection at 3,6 and 12 months. As the follow up increases, the number of VTE events will increase and that is expected to allow further exploration of the association of the studied biomarkers, clinical risk factors and disease specific parameters with VTE occurrence. The study of how hypercoagulability changes over time in the MM patient in association with the type of treatment but also in relation to treatment response will provide further insight to the underlying pathogenesis of hyper-coagulability in MM patients. Data on larger cohorts of patients with SMM and MGUS will allow us to assess how biomarkers of coagulation are linked to progression to MM in some of the patients.

5 Conclusions:

Venous thromboembolism remains a significant complication for patients with a diagnosis of multiple myeloma associated with considerable additional morbidity. Currently, the IMWG recommends thromboprophylaxis for patients with NDMM on IMiDs for the 4-6 first months of treatment or for as long as the risk of thrombosis remains high and the choice of prophylaxis medication depends on baseline risk stratification. Patients with 0 or 1 risk factors should receive aspirin (80-325 mg) and patients with 2 or more risk factors prophylactic dose LMWH. (445) A clear recommendation for MM patients on non-IMiD based treatment is lacking. IMWG, EMN and NCCN guidelines are all based on the limited data available from RCTs. The extent to which they are being applied in everyday clinical practice is questionable according to some recent reports, as clinicians tend to opt for thromboprophylaxis based more on personal perception of thrombotic risk. (363) (430) Even when recommendations are being applied residual VTE rates remain significant pointing to the need for optimization of current guidelines.(342, 430) More sensitive risk stratification tools are required to reflect more accurately all aspects of the procoagulant environment that exists in patients with MM.

The complexities of MM as a disease makes it very challenging to understand and comprehensively describe how coagulation pathways are altered within the MM microenvironment to result in a prothrombotic state. The multiplicity of the parameters that affect coagulation results in a unique coagulation profile for almost each myeloma patient. Advances in the field have not been major in recent years and no break-through data have been presented. Attempts by various study groups are however directed in the right direction to address unanswered questions and unmet needs. The first and most important step is to improve risk stratification of MM patients using more sensitive tools. A Risk assessment model developed specifically to assess risk in MM patients is required. Various groups have recently presented clinical risk scores but these have not been prospectively validated.

In an attempt to capture the unique procoagulant profile of the myeloma patient efforts have also focused in the identification of biomarkers of plasma or cellular hypercoagulability that are associated with increased VTE risk in MM patients that can be incorporated into clinical RAMs. So far no group has established a clear link between increased risk of thrombosis and a generic marker of coagulation. Such a task is demanding given the complex and heterogeneous coagulation profile of the myeloma patient. To allow application and incorporation in every day clinical practice biomarkers need to be easily assessed with no extra cost associated or expertise requirements.

At the same time there is an ever increasing need for RCTs designed to compare modes of thromboprophylaxis in MM patients based on the clear cut risk stratification criteria in order to provide robust data for optimum management of this significant complication in MM patients. Emerging data from ongoing trials that assess the role of DOACs in the prophylaxis of thrombosis in MM are of great importance. More RCTs need to be designed to assess their use versus LMWH in high-risk MM patients and versus aspirin in low risk MM patients. Data are required for both newly diagnosed and refractory/relapsed patients and for patients on non-IMiD containing regimens. There is lack of robust clinical data on the safety and efficacy of thromboprophylaxis agents on which to base updated IMWG and EMN recommendations and efforts in the field should focus on providing such data. DOACs are a convenient and cost-effective alternative to LMWH and warfarin and if robust data that supports their efficacy and safety emerges their use can be incorporated easily in daily clinical practice. Their use in the frail older patient, in the patient with impaired renal function and thrombocytopenia also needs to be assessed particularly in the context of the myeloma patient.

In the next 5 to 10 years hopefully the rates of this significant complication associated with MM will have been minimized. The means of achieving that is the development of a RAM and incorporates clinical risk factors, treatment-related risk factors and markers that reflect the underlying pro-coagulant environment. New guidelines will be available and incorporated into everyday clinical practice. DOACs will have probably substituted the use of LMWH and warfarin for the majority of patients.

Our study demonstrates the presence of pronounced cellular hypercoagulability in newly diagnosed chemotherapy naïve patients with symptomatic multiple myeloma, characterized by decreased Procoag-PPL® clotting time, enhanced endothelial cell activation, and exhausted thrombin generation. Coagulation abnormalities are also present in the precursor disease states of SMM and MGUS. Among a large number of biomarkers of hypercoagulability the Procoag-PPL clotting time and the ETP of thrombin generation were found to be independently associated with the risk of VTE. The new score formulated accurately stratifies patients to high and intermediate/low level of VTE risk. This score needs to be prospectively validated. Once validated it should be incorporated into a RAM that also includes clinical risk factors, disease specific and treatment specific parameters to increase its sensitivity and specificity in the risk stratification of patients. It is the aim of the ongoing ROADMAP-MM-CAT study to develop such a tool which should then be taken into consideration when designing phase III clinical trials that evaluate the efficacy and safety of pharmacological thromboprophylaxis in outpatients with multiple myeloma.

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Publications

1. Maria Gavriatopoulou, Ioannis Ntanasis-Stathopoulos, Lia-Angela Moulopoulos, Alexandros Manaios, **Despina Fotiou**, Evangelos Eleutherakis-Papaiakovou, Magdalini Migkou, Charis Bourgioti, Evangelos Terpos, Efstathios Kastritis, Meletios-Athanasios Dimopoulos. Treatment of Bing–Neel syndrome with first line sequential chemoimmunotherapy: A case report. Medicine. 2019;98(44):17794.

2. Evangelos Terpos, Ioannis V Kostopoulos, Efstathios Kastritis, Ioannis Ntanasis-Stathopoulos, Magdalini Migkou, Pantelis Rousakis, Alexandra T Argyriou, Nikolaos Kanellias, **Despina Fotiou,** Evangelos Eleutherakis-Papaiakovou, Maria Gavriatopoulou, Dimitrios C Ziogas, Aristea-Maria Papanota, Marilyn Spyropoulou-Vlachou, Ioannis P Trougakos, Ourania E Tsitsilonis, Bruno Paiva, Meletios A Dimopoulos. Impact of Minimal Residual Disease Detection by Next-Generation Flow Cytometry in Multiple Myeloma Patients with Sustained Complete Remission after Frontline Therapy. HemaSphere. 2019; doi: 10.1097/HS9.0000000000000300

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34. Dimitrios C. Ziogas, Efstathios Kastritis, Evangelos Terpos, Maria Roussou, Magdalini Migkou, Maria Gavriatopoulou, Despoina Spanomichou Evangelos Eleutherakis-Papaiakovou, **Despoina Fotiou**, Ioannis Panagiotidis, Eftychia Kafantari, Erasmia Psimenou, Ioannis Boletis, Demetrios V. Vlahakos, Harikleia Gakiopoulou, Charis Matsouka, Meletios A. Dimopoulos Hematologic and Renal Improvement of Monoclonal Immunoglobulin Deposition Disease after treatment with bortezomib-based regimens. Leuk Lymphoma. 2016 Dec 14:1-8

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Reviewer in international journals

- **Cancer control**
- **Expert Review of Hematology**
- **Hematology**

Poster Presentations

1. Maria Gavriatopoulou, Ioannis Ntanasis-Stathopoulos, Lia-Angela Moulopoulos, Alexandros Manaios, **Despina Fotiou,** Charis Bourgioti, Evangelos Terpos , Efstathios Kastritis, Meletios A. Dimopoulos. TREATMENT OF BING-NEEL SYNDROME WITH FIRST LINE SEQUENTIAL CHEMO-IMMUNOTHERAPY. P01. Presented in: 6th Aegean Hematology Oncology Symposium (2019), Cesme-Izmir, Turkey, 3-6 October 2019

2. Evangelos Terpos, Ioannis Ntanasis-Stathopoulos, Maria Roussou, Nikolaos Kanellias, **Despina Fotiou**, Magdalini Migkou, Evangelos Eleutherakis-Papaiakovou, Maria Gavriatopoulou, Efstathios Kastritis, Meletios A. Dimopoulos. FUNCTIONAL CARE IN THE ERA OF CONVENTIONAL CHEMOTHERAPY AND OF FIRST-GENERATION NOVEL ANTI-MYELOMA AGENTS; THE 20-YEAR EXPERIECNE OF A REFERRAL CENTER. P02. Presented in: 6th Aegean Hematology Oncology Symposium (2019), Cesme-Izmir, Turkey, 3-6 October 2019

3. Nikolaos Kanellias, Efstathios Kastritis, Maria Gavriatopoulou, Ioannis Ntanasis-Stathopoulos, Aristea-Maria Papanota, **Despina Fotiou,** Magdalini Migkou, Evangelos Eleutherakis-Papaiakovou, Panagiotis Maladrakis, Tina Bagratuni, Maria Roussou, Meletios A. Dimopoulo, Evangelos Terpos. NATURAL HISTORY OF SKELETAL RELATED EVENTS IN PATIENTS WITH MULTIPLE MYELOMA WHO RECEIVED FIRST- AND SECOND- LINE THERAPY WITH NOVEL AGENTS: A SINGLE CENTER ANALYSIS IN 620 PATIENTS. P05. Presented in: 6th Aegean Hematology Oncology Symposium (2019), Cesme-Izmir, Turkey, 3-6 October 2019

4. Evangelos Terpos, Maria Roussou, Efstathios Kastritis, Alexandra Margeli, Evangelos Eleutherakis-Papaiakovou, Ioannis Ntanasis-Stathopoulos, Maria Roussou, Nikolaos Kanellias, Gerasimos-Petros Papasotiriou, **Despina Fotiou**, Magdalini Migkou, Maria Gavriatopoulou, Ersasmia Psimenou, Ioannis Papasotiriou, Meletios A. Dimopoulos. SERUM NEUTROPHIL GELATINASE ASSOCIATED LIPOCALIN INDEPENDENTLY PREDICTS FOR RENAL RESPONSE IN MYELOMA PATIENTS WITH SEVERE RENAL IMPAIRMENT. P07 Presented in: 6th Aegean Hematology Oncology Symposium (2019), Cesme-Izmir, Turkey, 3-6 October 2019

5. Evangelos Eleutherakis-Papaiakovou, Ioannis V. Kostopoulos, Aristea-Maria Papanota, Paraskevi Micheli, Panagiotis Malandrakis, Nikolaos Kanellias, Ioannis Ntanasis-Stathopoulos, **Despina Fotiou**, Magdalini Migkou, Christine-Ivy Liacos, Maria Gavriatopoulou, Efstathios Kastritis, Ourania Tsitsilonis, Meletios A. Dimopoulos, Evangelos Terpos. P20. ABSENCE OF ABERRANT PLASMA CELLS IN THE APHERESIS PRODUCT PREDICTS FOR MINIMAL RESIDUAL DISEASE NEGATIVITY AFTER

AUTOLOGOUS TRANSPLANTATION IN MYELOMA PATIENTS WHO RECEIVE FIRST LINE THERAPY. Presented in: 6th Aegean Hematology Oncology Symposium (2019), Cesme-Izmir, Turkey, 3-6 October 2019

6. Ioannis V. Kostopoulos, Efstathios Kastritis, Aristea-Maria Papanota, Paraskevi Micheli, Panagiotis Malandrakis, Ioannis Ntanasis-Stathopoulos, **Despina Fotiou**, Nikolaos Kanellias, Magdalini Migkou, Evangelos Eleutherakis-Papaiakovou, Maria Gavriatopoulou, Ourania Tsitsilonis, Meletios A. Dimopoulos, Evangelos Terpos. MINIMAL RESIDUAL DISEASE IN PATIENTS WITH MULTIPLE MYELOMA WHO ACHIEVE COMPLETE RESPONSE AFTER FIRST LINE THERAPY. P22 Presented in: 6th Aegean Hematology Oncology Symposium (2019), Cesme-Izmir, Turkey, 3-6 October 2019

7. Efstathios Kastritis, Maria Roussou, Charikleia Gakiopoulou, Erasmia Psimenou, Maria Gavriatopoulou, Magdalini Migkou, Nikolaos Kanellias, Ioanna Dialoupi, Dimitrios C. Ziogas, Evangelos Eleutherakis-Papaiakovou, **Despina Fotiou,** Aristea-Maria Papanota, Stavroula Giannouli, Anastasia Pouli, Zafeirios Kartasis5, Christina Delavinia, Kostantinos Efstathiou, Ioanna Tatouli, Fotios Michas, Sofoklis Kontogiannis1, Evangelos Terpos, Meletios A Dimopoulos. P25. Carfilzomib-Associated Renal Toxicity Is Common and Unpredictable: An Analysis of 114 Patients. Presented in: 6th Aegean Hematology Oncology Symposium (2019), Cesme-Izmir, Turkey, 3-6 October 2019

8. Efstathios Kastritis, Aggeliki Laina, Maria Gavriatopoulou, Georgios Georgiopoulos, Nikolaos Makris, Evangelos Eleutherakis-Papaiakovou, **Despoina Fotiou,** Nikolaos Kanellias, Ioanna Dialoupi, Efstathios Manios, Magdalini Migkou, Dimitrios C. Ziogas, Maria Roussou, Aristea-Maria Papanota, Eleni-Dimitra Papanagnou, Maria Kotsopoulou, Anastasia Pouli, Evangelos Terpos, Ioannis P. Trougakos, Kimon Stamatelopoulos, Meletios A. Dimopoulos. FLOW-MEDIATED DILATATION AND AORTIC BLOOD PRESSURE PREDICT CARDIOVASCULAR ADVERSE EVENTS DURING CARFILZOMIB TREATMENT: A PROSPECTIVE STUDY IN PATIENTS WITH RELAPSED/ REFRACTORY MULTIPLE MYELOMA. Presented in: 6th Aegean Hematology Oncology Symposium (2019), Cesme-Izmir, Turkey, 3-6 October 2019

9. Efstathios Kastritis, Maria Gavriatopoulou, Ioannis V Kostopoulos, Ioanna Dialoupi, Maria Roussou, Nikolaos Kanellias, **Despoina Fotiou,** Evangelos Eleutherakis-Papaiakovou, Ioannis Ntanasis-Stathopoulos, Magdalini Migkou, Dimitrios C. Ziogas, Aristea-Maria Papanota, Asimina Papanikolaou[3] , Charikleia Gakiopoulou, Erasmia Psimenou, Maria Eirini Tselegkidi, Ourania Tsitsilonis, Ioannis Trougakos, Evangelos Terpos, Meletios A. Dimopoulos. CONSOLIDATION WITH A SHORT COURSE OF DARATUMUMAB CAN SIGNIFICANTLY IMPROVE COMPLETE RESPONSE RATES IN

PATIENTS WITH AL AMYLOIDOSIS OR LCDD. P28. Presented in: 6th Aegean Hematology Oncology Symposium (2019), Cesme-Izmir, Turkey, 3-6 October 2019

10. Evangelos Terpos, Ioannis V. Kostopoulos, Aristea-Maria Papanota, Konstantinos Papadimitriou, Panagiotis Malandrakis, Paraskevi Micheli, Ioannis Ntanasis-Stathopoulos, **Despina Fotiou**, Andreas Metousis, Nikolaos Kanellias, Magdalini Migkou, Evangelos Eleutherakis-Papaiakovou, Maria Gavriatopoulou, Efstathios Kastritis, Ourania E. Tsitsilonis, Meletios A. Dimopoulos. NEXT GENERATION FLOW CYTOMETRY PROVIDES A HIGHLY SENSITIVE METHOD FOR THE EVALUATION OF CIRCULATING PLASMA CELLS IN NEWLY DIAGNOSED MULTIPLE MYELOMA: A SINGLE CENTER STUDY IN 182 PATIENTS. P32. Presented in: 6th Aegean Hematology Oncology Symposium (2019), Cesme-Izmir, Turkey, 3-6 October 2019
11. Evangelos Terpos, Konstantinos Anargyrou, **Despina Fotiou,** Theodoros P. Vassilakopoulos, Dimitrios Christoulas, Maria Dimou, Ioannis Ntanasis-Stathopoulos, Stavroula Masouridou, Athanasios Papatheodorou, Konstantinos Tsionos, Panayiotis Panayiotidis, Meletios A. Dimopoulos. LOW BONE MINERAL DENSITY AND HIGH BONE TURNOVER in PATIENTS with NON-HODGKIN'S LYMPHOMA who RECEIVE FRONTLINE THERAPY: RESULTS of a MULTICENTER PROSPECTIVE STUDY. P34. Presented in: 6th Aegean Hematology Oncology Symposium (2019), Cesme-Izmir, Turkey, 3-6 October 2019

12. Efstathios Kastritis, Despoina Spanomihou, Maria Gavriatopoulou, Maria Roussou, Evangelos Eleutherakis-Papaiakovou, Ioannis Ntanasis-Stathopoulos, Nikolaos Kanellias, **Despina Fotiou**, Magdalini Migkou, Evangelos Terpos, Meletios A. Dimopoulos. INCIDENCE AND RISK FACTORS FOR SECOND PRIMARY MALIGNANCIRS IN MULTIPLE MYELOMA. A SINGLE CENTER ANALYSIS OF 595 PATIENTS. P35. Presented in: 6th Aegean Hematology Oncology Symposium (2019), Cesme-Izmir, Turkey, 3-6 October 2019

13. Efstathios Kastritis, Maria Gavriatopoulou, Ioannis V Kostopoulos, Ioanna Dialoupi, Maria Roussou, Nikolaos Kanellias, **Despina Fotiou,** Evangelos Eleutherakis-Papaiakovou, Ioannis Ntanasis-Stathopoulos, Magdalini Migkou, Aristea-Maria Papanota, Asimina Papanikolaou, Charikleia Gakiopoulou, Erasmia Psimenou, Maria Eirini Tselegkidi, Ourania Tsitsilonis, Ioannis Trougakos, Evangelos Terpos, Meletios A. Dimopoulos. FOUR WEEKLY INFUSIONS OF DARATUMUMAB CONSOLIDATION SIGNIFICANTLY IMPROVE COMPLETE RESPONSE RATES IN PATIENTS WITH AL AMYLOIDOSIS OR LCDD. P40. Presented in: 6th Aegean Hematology Oncology Symposium (2019), Cesme-Izmir, Turkey, 3-6 October 2019

14. Efstathios Kastritis, Eleni A Karatrasoglou, Ioanna Dialoupi, Maria Gavriatopoulou, Maria Roussou, Nikolaos Kanellias, **Despina Fotiou**, Nikolaos Kanellias, Ioannis

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51. Maria Gavriatopoulou, Ioannis Ntanasis-Stathopoulos, Nick Kanellias, Magdalini Migkou, **Despoina Fotiou,** Maria Roussou, Efstathios Kastritis, Evangelos Terpos, Meletios A. Dimopoulos. The Addition of Pomalidomide to Daratumumab Achieves Remission for a Pentarefractory Patient: a Successful Story. Presented in: 4th Aegean Hematology Oncology Symposium (2017), Rhodes, Greece, 21-24 September 2017

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53. Ioannis Ntanasis-Stathopoulos, Maria Gavriatopoulou, Dimitrios C Ziogas, Magdalini Migkou, **Despoina Fotiou,** Nikolaos Kanellias, Evaggelos Eleutherakis-Papaiakovou, Efstathios Kastritis, Evangelos Terpos, Meletios-Athanasios Dimopoulos. Testicular plasmacytomas; the blood-testis barrier under question. Presented in: 4th Aegean Hematology Oncology Symposium (2017), Rhodes, Greece, 21-24 September 2017

54. Dimitrios C. Ziogas, Evangelos Terpos, Maria Gavriatopoulou, Magdalini Migkou, **Despoina Fotiou,** Nikolaos Kanellias, Ioannis Ntanasis-Stathopoulos, Evangelos Eleutherakis- Papaiakovou, Efstathios Kastritis, Meletios A. Dimopoulos. COEXISTENCE OF LEISHMANIASIS AND MULTIPLE MYELOMA IN THE ERA OF MONOCLONAL ANTIBOBY (ANTI-CD38 OR ANTI-SLAMF7) CONTAINING TRIPLETS: ONE SHARED STORY OF TWO EXCEPTIONAL CASES. Presented in: 4th Aegean Hematology Oncology Symposium (2017), Rhodes, Greece, 21-24 September 2017

55. Efstathios Kastritis, Giampaolo Merlini, Ioannis Papassotiriou, Paolo Milani, Evangelos Terpos, Marco Basset, Athanassios Akalestos, Francesca Russo, Erasmia Psimenou, Filia Apostolakou, Maria Roussou, **Maria Gavriatopoulou**, Despina Fotiou, Dimitrios Ziogas, Constantinos Pamboucas, Elektra Papadopoulou, Meletios A. Dimopoulos and Giovanni Palladini. Growth Differentiation Factor-15 (GDF-15) is a New Biomarker With Independent Prognostic Significance For Survival and Renal Outcomes in Different Cohorts of Patients with Light Chain (AL) Amyloidosis ASH 3-6 December 2016, San Diego, California

56. Meletios A. Dimopoulos, Maria Roussou, Maria Gavriatopoulou, **Despina Fotiou**, Dimitrios Ziogas, Magdalini Migkou, Ioannis Panagiotidis, Evangelos Eleutherakis-Papaiakovou, Nikolaos Kanellias, Erasmia Psimenou, John N Boletis, Dimitra Bacharaki, Despoina Mparmparoussi, Charis Matsouka, Stavroula Giannouli, Evangelos Terpos and Efstathios Kastritis Outcomes of Newly Diagnosed Myeloma Patients Requiring Dialysis: Dialysis Independence Is Associated with Rapid Myeloma Response and Predicts for Longer Survival ASH 3-6 December 2016, San Diego, California

57. Meletios A. Dimopoulos, Maria Roussou, Maria Gavriatopoulou, Erasmia Psimenou, Dimitrios Ziogas, Evangelos Eleutherakis-Papaiakovou, **Despina Fotiou**, Magdalini Migkou, Nikolaos Kanellias, Ioannis Panagiotidis, Argiris Ntalianis, Evangelos Repasos, Elektra Papadopoulou, Kimon Stamatelopoulos, Efstathios Manios, Constantinos Pamboukas, Sofoklis Kontogiannis, Evangelos Terpos and Efstathios Kastritis Cardiac and Renal Complications of Carfilzomib Therapy in Patients with Multiple Myeloma ASH 3-6 December 2016, San Diego, California

58. Meletios A. Dimopoulos, Maria Roussou, Nikolaos Kanellias, Maria Gavriatopoulou, Magdalini Migkou, Ioannis Panagiotidis, Evangelos Eleutherakis-Papaiakovou, Dimitrios Ziogas, **Despina Fotiou,** Stavroula Giannouli, Panagiotis Tsirigotis, Christos Poziopoulos, Sossana Delimpasi, Despoina Mparmparoussi, Charis Matsouka, Kostas Konstantopoulos, Evangelos Terpos and Efstathios Kastritis Pomalidomide with Low Dose Dexamethasone Is Effective Irrespective of Primary or Secondary Resistance to Lenalidomide but the IMiD-Free Interval Is Important ASH 3-6 December 2016, San Diego, California

59. Efstathios Kastritis, Maria Gavriatopoulou, Maria Roussou, **Despina Fotiou,** Dimitrios Ziogas, Magdalini Migkou, Evangelos Eleutherakis Papaiakovou, Ioannis Panagiotidis, Erasmia Psimenou, Elektra Papadopoulou, Constantinos Pamboucas, Efstathios Manios, Harikleia Gakiopoulou, Anna Tasidou, Stavroula Giannouli, Evangelos Terpos, and Meletios A. Dimopoulos Addition of Cyclophosphamide and Higher Doses of Dexamethasone Do Not Improve Outcomes of Patients with AL Amyloidosis Treated with Bortezomib ASH 3-6 December 2016, San Diego, California

60. M. Gavriatopoulou, E. Kastritis, **D. Fotiou**, D. Ziogas, E. Terpos, M. Roussou and M.A Dimopoulos Phase 2 study of Ofatumumab, Fludarabine and Cyclophosphamide in Relapsed/Refractory Waldenstrom Macroglobulinemia, 9th International Workshop on Waldenstrom's Macroglobulinemia, 5-8 October 2016, Amsterdam, The Netherlands

61. Maria Gavriatopoulou, Efstathios Kastritis, Evangelos Terpos, Magdalini Migkou, Evangelos Eleutherakis-Papaiakovou, Dimitrios Ziogas, Ioannis Panagiotidis, Maria Roussou, **Despoina Fotiou** and Meletios A. Dimopoulos Evaluation of the Revised International Staging System (R-ISS) in an Independent Cohort of Unselected Patients with Multiple Myeloma, Society of Hematologic Oncology, Houston, Texas, 6-10 September 2016

62. E Kastritis, M Gavriatopoulou, M Roussou, E Terpos, **D Fotiou**, DC Ziogas, E Eleutherakis-Papaiakovou, C Pamboukas, E Papadopoulou, E Psimenou, K Stamatelopoulos, F Michas, A Lykka, MA Dimopoulos and E Manios Prospective Evaluation of the Clinical and Prognostic Implications of Blood Pressure Monitoring and Baroreceptor Reflex Sensitivity (BRS) in Patients with AL Amyloidosis The XVth International Symposium on Amyloidosis, Uppsala, 3–7 July 2016, Abstract PC24

63. E Kastritis, DC Ziogas, M Gavriatopoulou, E Terpos, M Roussou, M Migkou, E Eleutherakis-Papaiakovou, **D Fotiou,** I Panagiotidis E Kafantari, E Psimenou, I Boletis, DV Vlahakos, H Gakiopoulou, C Matsouka, MA Dimopoulos Hematologic and renal improvement of monoclonal immunoglobulin deposition disease after treatment with bortezomib-based regimens The XVth International Symposium on Amyloidosis, Uppsala, 3–7 July 2016, Abstract PC25

64. M Gavriatopoulou, M Roussou, E Terpos, **D Fotiou,** DC Ziogas, E Eleutherakis-Papaiakovou, T Apostolou, I Boletis, C Pamboukas, E Papadopoulou, E Psimenou, MA Dimopoulos, E Kastritis Importance of renal stage, treatment and hematologic response in the risk of end stage renal disease in patients with AL amyloidosis The XVth International Symposium on Amyloidosis, Uppsala, 3–7 July 2016, Abstract PC27

65. E Kastritis, M Gavriatopoulou, G Georgiopoulos, M Roussou, D Ziogas, **D Fotiou,** E Terpos, M Lykka, F Athanasouli, C Papamichail, MA Dimopoulos, K Stamatelopoulos Vascular dysfunction in AL amyloidosis: prospective evaluation of markers of vascular involvement and their clinical significance The XVth International Symposium on Amyloidosis, Uppsala, 3–7 July 2016, Abstract PC28

66. E Terpos, E Kastritis, D Christoulas, E Eleutherakis-Papaiakovou, M Gavriatopoulou**, D Fotiou,** I Panagiotidis, D Ziogas, E Kafantari, M Migkou, M Roussou, MA Dimopoulos Consolidation with bortezomib and lenalidomide post-ASCT without dexamethasone and bisphosphonates: final analysis of a prospective study in newly diagnosed myeloma patients 21st Congress of the European Hematology Association, Copenhagen June 9 - 12, 2016, Abstract P284

67. MA Dimopoulos, M Gavriatopoulou, M Roussou, **D Fotiou**, D Ziogas, M Migkou, I Panagiotidis, S Orfanopoulos, U Koloventzou, V Babali, E Kafantari, S Giannouli, E Eleutherakis-Papaiakovou, K Konstantopoulos, P Tsirigotis, E Terpos, E Kastritis Prospective evaluation of geriatric assessment tools in real-world, unselected, elderly patients with symptomatic myeloma 21st Congress of the European Hematology Association, Copenhagen June 9 - 12, 2016, Abstract P657

68. M Gavriatopoulou, M Roussou, **D Fotiou,** D Ziogas, E Terpos, D Kalapanida, E Kafantari, A Papathoma, M Spyropoulou-Vlachou, MA Dimopoulos, E Kastritis A phase 2 study of the combination of ofatumumab with fludarabine and cyclophosphamide in patients with relapsed or refractory Waldenström's macroglobulinemia 21st Congress of the European Hematology Association, Copenhagen June 9 - 12, 2016, Abstract E1155

69. D Ziogas, M Roussou, **D Fotiou,** E Terpos, M Gavriatopoulou, E Eleutherakis-Papaiakovou, E Psimenou, I Boletis, M Spyropoulou-Vlachou, C Gakiopoulou, MA Dimopoulos, E Kastritis Renal and hematologic outcomes of bortezomib-based treatment in patients with light chain deposition disease 21st Congress of the European Hematology Association, Copenhagen June 9 - 12, 2016, Abstract E1294

70. E Kastritis, M Roussou, M Gavriatopoulou, **D Fotiou,** D Ziogas, K Stamatelopoulos, C Pamboucas, E Papadopoulou, F Michas, A Lykka, E Terpos, S Giannouli, MA Dimopoulos, E Manios Prospective evaluation of prognostic and pathophysiologic implications of blood pressure monitoring and baroreceptor reflex sensitivity (BRS) in patients with al amyloidosis 21st Congress of the European Hematology Association, Copenhagen June 9 - 12, 2016, Abstract E1306

71. Efstathios Kastritis, Evangelos Terpos, Nikolaos Kanellias, Vasiliki Babali, Spyridon Orfanopoulos, Ursula Koloventzou, Maria Gavriatopoulou**, Despoina Fotiou,** Dimitrios Ziogas, Eftychia Kafantari, Stavroula Giannouli, Meletios A. Dimopoulos Real-World Prospective Evaluation of Different Geriatric Assessment Tools in Unselected Elderly Patients with Symptomatic Myeloma 57th America Society of Hematology, Orlando, 5-7 Δεκεμβρίου, 2015; Abstract 4242

72. Efstathios Kastritis, Evangelos Terpos, Maria Roussou, Vasilis Koutoulidis, Stavroula Giannouli, Maria Gavriatopoulou, Magdalini Migkou, Evangelos Eleutherakis-Papaiakovou, **Despoina Fotiou,** Dimitrios Ziogas, Ioannis Panagiotidis, Despoina Kalapanida, Eirini Katodritou, Kostas Konstantopoulos, Lia A. Moulopoulos, Meletios A. Dimopoulos Validation of the Novel Criteria for the Definition of Symptomatic Myeloma: A Single Center Experience in 216 Patients with the Previous Diagnosis of

Asymptomatic Disease 57th America Society of Hematology, Orlando , 5-7 Δεκεμβρίου, 2015; Abstract 4251

73. Meletios A. Dimopoulos, Efstathios Kastritis, Maria Roussou, Erasmia Psimenou, Maria Gavriatopoulou, Magdalini Migkou, Evangelos Eleutherakis-Papaiakovou, Dimitrios Ziogas, Ioannis Panagiotidis, **Despoina Fotiou,** Eftychia Kafantari, Stavroula Giannouli, Sofoklis Kontogiannis, Evangelos Terpos Bortezomib-Based Triplets Are Associated with a High Probability of Dialysis Independence and Rapid Renal Recovery in Newly Diagnosed Myeloma Patients with Severe Renal Failure or Those Requiring Dialysis 57th America Society of Hematology, Orlando , 5-7 Δεκεμβρίου, 2015; Abstract 1832

74. Efstathios Kastritis, Maria Roussou, **Despoina Fotiou**, Dimitrios Ziogas, Maria Gavriatopoulou**,** Kimon Stamatelopoulos, Constantinos Pamboucas, Elektra Papadopoulou, Fotios Michas, Aikaterini Likka, Evangelos Terpos, Meletios A. Dimopoulos, Efstathios Manios Prospective Evaluation of Blood Pressure Monitoring and Baroreceptor Reflex Sensitivity (BRS) in Patients with AL Amyloidosis: Prognostic and Pathophysiologic Implications 57th America Society of Hematology, Orlando , 5-7 Δεκεμβρίου, 2015; Abstract 3054

75. Efstathios Kastritis, Evangelos Terpos, Maria Gavriatopoulou, Magdalini Migkou, Evangelos Eleutherakis-Papaiakovou, Dimitrios Ziogas, Ioannis Panagiotidis, **Despoina Fotiou**, Dimitra Gika, Despoina Kalapanida, Eftychia Kafantari, Maria Roussou, Flora Zagouri, Stavroula Giannouli, Athanasios Zomas, Kostas Konstantopoulos, Meletios A. Dimopoulos Evaluation of the Revised International Staging System (R-ISS) in an Independent Cohort of Unselected Patients with Multiple Myeloma 57th America Society of Hematology, Orlando , 5-7 Δεκεμβρίου, 2015; Abstract 3045

76. E. Terpos, D. Christoulas, E. Kastritis, E. Eleutherakis-Papaiakovou, M. Gavriatopoulou, D. Ziogas, M. Migkou, I. Panagiotidis, D. Kalapanida, **D. Fotiou,** E. Kafantari, F. Zagouri, M.A. Dimopoulos Sclerostin Remains Elevated Even in the Plateau Phase of Myeloma Patients: Implications into the Pathogenesis of Osteoblast Dysfunction of Multiple Myeloma 15th International Myeloma Workshop, Rome, 23-26th September, 2015; Abstract PO-257

Participation as a Sub-Investigator in International Clinical Trials and Research Projects

- **A Phase 3 (EMN18) A multicenter, open label, randomized phase ii study comparing daratumumab combined with bortezomib-cyclophosphamide-dexamethasone (dara-vcd) versus the association of bortezomib-thalidomide-**

dexamethasone (vtd) as pre transplant induction and post transplant consolidation, both followed by a maintenance phase with ixazomib alone or in combination with daratumumab, in newly diagnosed multiple myeloma (mm) young patients eligible for autologous stem cell transplantation.
Principal Investigator: Professor MA Dimopoylos

- **A Phase 3 (EMN17/54767414MMY3014) Daratumumab, VELCADE (Bortezomib), Lenalidomide and Dexamethasone Compared to VELCADE, Lenalidomide and Dexamethasone in Subjects With Previously Untreated Multiple Myeloma (Perseus).**
  Principal investigator: Professor MA Dimopoulos
- **A Study Comparing Once-weekly vs Twice-weekly Carfilzomib in Combination With Lenalidomide and Dexamethasone in Subjects With Relapsed or Refractory Multiple Myeloma (ARROW2)**
  Principal investigator: Professor MA Dimopoulos
- **Phase 2 study of daratumumab monotherapy in previously untreated patients with stage 3B light chain (AL) amyloidosis (EMN22/54767414AMY2005)**
  Principal Investigator: Professor Efstathios Kastritis
- **A Phase 3 Randomized, Open-label, Multicenter Study Assessing the Clinical Benefit of Isatuximab (SAR650984) in Combination With Bortezomib (Velcade®), Lenalidomide (Revlimid®) and Dexamethasone Versus Bortezomib, Lenalidomide and Dexamethasone in Patients With Newly Diagnosed Multiple Myeloma (NDMM) Not Eligible for Transplant (IMROZ)**
  Principal investigator: Professor MA Dimopoulos
- **Randomized, Open Label, Multicenter Study Assessing The Clinical Benefit Of Isatuximab Combined With Carfilzomib (Kyprolis) And Dexamethasone Versus Carfilzomib With Dexamethasone In Patients With Relapse And/Or Refractory Multiple Myeloma Previously Treated With 1 to 3 Prior Lines (IKEMA)**
  Principal investigator: Professor MA Dimopoulos
- **A Randomized, Open-label, Phase 3 Study Comparing Carfilzomib, Dexamethasone, and Daratumumab to Carfilzomib and Dexamethasone for the Treatment of Patients With Relapsed or Refractory Multiple Myeloma (CANDOR Study)**
  Principal investigator: Professor MA Dimopoulos
- **A Study of Subcutaneous Daratumumab Versus Active Monitoring in Participants With High-Risk Smoldering Multiple Myeloma (Aquila Study)**
  Principal investigator: Professor MA Dimopoulos
- **A Phase 3, Randomized, Placebo-Controlled, Double-Blind Study of Oral Ixazomib Maintenance Therapy After Initial Therapy in Patients With Newly Diagnosed Multiple Myeloma Not Treated With Stem Cell Transplantation (C16021)**
  Principal investigator: Professor MA Dimopoulos

- **A Phase 3 Study Comparing Daratumumab, Velcade, Lenalidomide and Dexamethasone (D-VRd) vs Velcade, Lenalidomide and Dexamethasone (VRd) in Subjects with Previously Untreated Multiple Myeloma who are Eligible for High-dose Therapy (EMN17/54767414MMY3014)**
  Principal investigator: Professor MA Dimopoulos

- **A Phase 3 Multicenter, Randomized, Open Label Study of Venetoclax and Dexamethasone Compared with Pomalidomide and Dexamethasone in Subjects with t(11;14) Positive Relapsed or Refractory Multiple Myeloma (The Canova Study)**
  Principal investigator: Professor MA Dimopoulos

- **Efficacy of Daratumumab in Patients With Relapsed/Refractory Myeloma With Renal Impairment (The DARE study)**
  Principal investigator: Associate Professor Efstathios Kastritis

- **Effects of Daratumumab Monotherapy on Bone Parameters in Patients With Relapsed and /or Refractory Multiple Myeloma (REBUILD)**
  Principal investigator: Professor Evangelos Terpos

- **A Phase 2, Open-Label Study of Ixazomib+ Daratumumab+ Dexamethasone (IDd) in Relapsed and/or Refractory Multiple Myeloma (RRMM) (C16047)**
  Principal investigator: Professor MA Dimopoulos

- **A Phase 2, Multicenter, Open-label, Single-Arm Study to Evaluate the Safety and Efficacy of Daratumumab in Combination with Ixazomib and Dexamethasone as Second Line Therapy in Multiple Myeloma Patients who have received prior treatment with a Lenalidomide based regimen. (The DARIA Study)**
  Principal investigator: Professor MA Dimopoulos

- **A Phase 1, Open-label Study to Evaluate the Safety, Pharmacokinetics, Immunogenity, and Preliminary Efficacy of MEDI2228 in Subjects with Relapsed/Refractory Multiple Myeloma (MedImmune)**
  Principal investigator: Professor MA Dimopoulos

- **Multiple Phase ½ Cohorts of Nivolumab Monotherapy or Nivolumab Combination Regimens Across Relapsed/Refractory Hematologic Malignancies**
  Principal investigator: Professor MA Dimopoulos

- **The APOLLO study 54767414MMY3013: A Phase 3 Study Comparing Pomalidomide and Dexamethasone With or Without Daratumumab in Subjects With Relapsed or**

Refractory Multiple Myeloma Who Have Received at Least One Prior Line of Therapy With Both Lenalidomide and a Proteasome Inhibitor.
Principal Investigator: Prof. Meletios-Athanasios Dimopoulos

- **EFC14335 (ICARIA-MM): A phase 3 randomized, open-label, multicenter study comparing Isatuximab (SAR650984) in Combination with pomalidomide And low-dose dexamethasone veRsus pomalidomide and low-dose dexamethasone In patients with refractory or relapsed And refractory Multiple Myeloma**

  Principal Investigator: Prof. Meletios-Athanasios Dimopoulos

- **Amgen Protocol 20150267: A Randomized, Open-label Phase 3 Study of Carfilzomib, Lenalidomide, and Dexamethasone versus Bortezomib, Lenalidomide and Dexamethasone in Transplant-eligible Patients With Newly Diagnosed Multiple Myeloma**
  Principal Investigator: Prof. Meletios-Athanasios Dimopoulos

- **CA209-602: An Open-Label, Randomized Phase 3 Trial of Combinations of Nivolumab, Elotuzumab, Pomalidomide and Dexamethasone in Relapsed and Refractory Multiple Myeloma- CheckMate 602: CHECKpoint Pathway and nivoluMAb Clinical Trial Evaluation 602**
  Principal Investigator: Prof. Meletios-Athanasios Dimopoulos

- **KCP-330-012 STORM: A Phase 2b, Open-Label, Single-Arm Study of Selinexor (KPT-330) Plus Low-Dose Dexamethasone (Sd) in Patients with Multiple Myeloma Previously Treated with Lenalidomide, Pomalidomide, Bortezomib, Carfilzomib, and an anti-CD38 Monoclonal Antibody (mAb) and Refractory to Prior Treatment with Glucocorticoids, an Immunomodulatory Agent, a Proteasome Inhibitor and an anti-CD38 mAb**
  Principal Investigator: Prof. Meletios-Athanasios Dimopoulos

- **MEDI4736-MM-005 MULTICENTER, SINGLE-ARM, PHASE 2 STUDY TO DETERMINE THE EFFICACY FOR THE COMBINATION OF DARATUMUMAB (DARA) PLUS DURVALUMAB (DURVA) (D2) IN SUBJECTS WITH RELAPSED AND REFRACTORY MULTIPLE MYELOMA (RRMM) WHO HAVE PROGRESSED ON DARA WHILE ON A DARA-CONTAINING REGIMEN AS THE MOST RECENT MULTIPLE MYELOMA THERAPY. "FUSION MM-005"**
  Principal Investigator: Prof. Meletios-Athanasios Dimopoulos

- **BGB-3111-302: A Phase 3, Randomized, Open-Label, Multicenter Study Comparing the Efficacy and Safety of the Bruton's Tyrosine Kinase (BTK) Inhibitors BGB-3111 and Ibrutinib in Subjects With Waldenström's Macroglobulinemia (WM)**
  Principal Investigator: Prof. Meletios-Athanasios Dimopoulos

- **OP-103 OCEAN: A Randomized, Controlled, Open-Label, Phase 3 Study of Melflufen/ Dexamethasone Compared with Pomalidomide/Dexamethasone for Patients with Relapsed Refractory Multiple Myeloma who are Refractory to Lenalidomide**
  Principal Investigator: Prof. Meletios-Athanasios Dimopoulos

- **CLBH589D2222 PANORAMA-3: A multicenter, randomized, open-label Phase 2 study evaluating the safety and efficacy of three different regimens of oral panobinostat in combination with subcutaneous bortezomib and oral dexamethasone in patients with relapsed or relapsed/refractory multiple myeloma who have been previously exposed to immunomodulatory agents**
  Principal Investigator: Prof. Meletios-Athanasios Dimopoulos

- **C16020: An Open-Label, Phase 2 Study to Evaluate the Oral Combination of MLN9708 With Cyclophosphamide and Dexamethasone In Patients With Newly Diagnosed or Relapsed and/or Refractory Multiple Myeloma Requiring Systemic Treatment**
  Principal Investigator: Prof. Meletios-Athanasios Dimopoulos

- **CC-4047-MM-013: A Phase 2 Multicenter, Open-label Study to Determine the Efficacy and Safety of Pomalidomide (CC-4047) in Combination With Low-Dose Dexamethasone in Subjects With Relapsed or Refractory Multiple Myeloma and Moderate or Severe Renal Impairment Including Subjects Undergoing Hemodialysis**
  Principal Investigator: Prof. Meletios-Athanasios Dimopoulos

- **54767414MMY3007 ALCYONE: A Phase 3, Randomized, Controlled, Open-label Study of VELCADE (Bortezomib) Melphalan-Prednisone (VMP) Compared to Daratumumab in Combination with VMP (D-VMP), in Subjects with Previously Untreated Multiple Myeloma who are Ineligible for High-dose Therapy**
  Principal Investigator: Prof. Meletios-Athanasios Dimopoulos

- **POLLUX: Phase 3 Study Comparing Daratumumab, Lenalidomide, and Dexamethasone (DRd) vs Lenalidomide and Dexamethasone (Rd) in Subjects with Relapsed or Refractory Multiple**
  Principal Investigator: Professor MA Dimopoulos

- **Phase 3 Clinical trial: ELOQUENT-1 «Phase III Study of Lenalidomide and Dexamethasone with or Without Elotuzumab to Treat Newly Diagnosed, Previously Untreated Multiple Myeloma (ELOQUENT - 1)**
  Principal Investigator: Professor MA Dimopoulos

- **Phase I/II Clinical trial: "HOVON 124 WM/ECWM-R2 study: A prospective phase I/II trial of the combination of ixazomib citrate, rituximab and dexamethasone in patients with relapsed or progressive Waldenström's macroglobulinemia: A HOVON/Greek Myeloma Study Group study"**
  Principal Investigator: Professor MA Dimopoulos
- **Phase 2 clinical Trial: ECWM1: Efficacy of first line Dexamethasone, Rituximab and Cyclophosphamide+/-Bortezomib for patients with Waldenstrom' s Macroglobulinemia**
  Principal Investigator: Professor MA Dimopoulos

Presentations in conferences

- Maria Gavriatopoulou, Evangelos Terpos, Ioannis Ntanasis-Stathopoulos, Panagiotis Malandrakis, Envagelos Eleutherakis-Papaiakovou, Athanasios Papatheodorou, Nikolaos Kanellias, Magdalini Migkou, **Despina Fotiou**, Ioanna Dialoupi, Maria Roussou, Nikoletta-Aikaterini Kokkali, Efstathios Kastritis, Meletios A. Dimopoulos. Newly diagnosed patients with multiple myeloma receiving ASCT and consolidation with carfilzomib, lenalidomide and dexemathasone (KRD) show high rates of minimal residual disease negativity. Presented in: 6th Aegean Hematology Oncology Symposium (2019), Cesme-Izmir, Turkey, 3-6 October 2019

- Aristea-Maria Papanota, Paraskevi Karousi, CHriston K. Kontos, Christine Liacos, Pinelopi I Artermaki, Dimitris Patseas, Aikaterini-Anna Liosi, Nefeli Mavrianou-Koutsoukou, Maria-Anna Kalioraki, **Despina Fotiou**, Ioannis-Ntanasis Stathopoulos, Magdalini Migkou, Maria Gavriatopoulou, Efstathios Kastritis, Andreas Scorilas, Meletios A. Dimopoulos, Evangelos Terpos. THREE TRNA-DERIVED RNA GRAGMENTS (3'-TRF—LEUAAG/TAG, I-TRF-GLUCTC AND I-TRF-PROTGG) DISCRIMINATE SMOLDERING FROM SYMPTOMATIC MULTIPLE MYELOMA PATIENTS. Presented in: 6th Aegean Hematology Oncology Symposium (2019), Cesme-Izmir, Turkey, 3-6 October 2019

- **Despina Fotiou**, Loula Papageorgiou, Stella Salta, Evangelos Terpos, Jawed Fareed, Patrick VanDreden, Annette K Larsen, Ismail Elalamy, Meletios A Dimopoulos[1], Grigoris Gerotziafas. **HYPERCOAGULABILITY BIOMARKERS IN A NEW SCORE LINKED TO TREATMENT RESISTANCE FOR MULTIPLE MYELOMA PATIENTS. THE ROADMAP-**

MM STUDY. Presented in: EMLTD 26th Anniversary International Congress on Thrombosis. 19-22 June 2019. Athens, Greece.

- **Despina Fotiou**, Theodoros N. Sergentanis, Loula Papageorgiou , Maria Gavriatopoulou, Theodora Psaltopoulou, Patrick VanDreden, Annette K Larsen, Ismail Elalamy Efstathios Kastritis, Kimon Stamatelopoulos, Evangelos Terpos, Meletios A. Dimopoulos, Grigoris T. Gerotziafas. **Prospective assessment of biomarkers of hypercoagulability for the identification of newly diagnosed chemotherapy naïve patients with multiple myeloma at risk for cancer-associated thrombosis. ROADMAT-CAT-MM STUDY.** Presented in: EMLTD 26th Anniversary International Congress on Thrombosis. 19-22 June 2019. Athens, Greece.

- Tina Bagratuni*, Ioannis Ntanasis-Stathopoulos*, Maria Gavriatopoulou, Nefeli Mavrianou-Koutsoukou, Christine Liacos, Dimitrios Patseas, Nikolaos Kanellias, Magdalini Migkou, Dimitrios C. Ziogas, Evangelos Eleutherakis-Papaiakovou, Maria Roussou, **Despina Fotiou,** Evangelos Terpos, Efstathios Kastritis, Meletios A. Dimopoulos. **DETECTION OF MYD88 AND CXCR4 MUTATIONS IN CELL-FREE DNA OF PATIENTS WITH IgM MONOCLONAL GAMMOPATHIES**. Presented in: 5th Aegean Hematology Oncology Symposium (2018), Porto Heli, Greece, 20-23 September 2018

- Ioannis Kostopoulos, Efstathios Kastritis, Ioannis Ntanasis-Stathopoulos, Magdalini Migkou, Alexandra T. Argyriou, Nikolaos Kanellias, **Despina Fotiou,** Evangelos Eleutherakis-Papaiakovou, Maria Gavriatopoulou, Aristea-Maria Papanota, Marilyn Spyropoulou-Vlachou3, Ourania E. Tsitsilonis, Bruno Paiva, Meletios A. Dimopoulos, Evangelos Terpos. **NEXT GENERATION FLOW CYTOMETRY FOR MINIMAL RESIDUAL DISEASE EVALUATION IN MULTIPLE MYELOMA PATIENTS WITH SUSTAINED COMPLETE RESPONSE AFTER FRONTLINE THERAPY: RESULTS OF A PROSPECTIVE SINGLE-CENTER ANALYSIS.** Presented in: 5th Aegean Hematology Oncology Symposium (2018), Porto Heli, Greece, 20-23 September 2018
- Efstathios Kastritis, Maria Roussou, Maria Gavriatopoulou, Tina Bagratuni, Magdalini Migkou, **Despina Fotiou,** Dimitrios C. Ziogas, Ioannis Ntanasis-Stathopoulos, Ioannis Panayiotidis, Nikolaos Kanellias, Alexandra Papathoma, Erasmia Psimenou, Charikleia Gakiopoulou, Smaragdi Marinaki, Elektra Papadopoulou, Argyrios Ntalianis, Evangelos Terpos, M.A. Dimopoulos. **Salvage lenalidomide-based therapy for patients with AL amyloidosis.** Presented in: ISA The XVI International Symposium on Amyloidosis (2018), Kumamoto, Japan, March 26-29, 2018

- Ioannis Ntanasis-Stathopoulos, Gerasimos-Petros Papassotiriou, Efstathios Kastritis, Alexandra Margeli, Nikolaos Kanellias, Evangelos Eleutherakis-Papaiakovou, Dimitrios Ziogas, **Despina Fotiou,** Magdalini Migkou, Maria Gavriatopoulou, Maria

Roussou, Erasmia Psimenou, Ioannis Papassotiriou, Meletios A. Dimopoulos, Evangelos Terpos. **Circulating Neutrophil Gelatinase-Associated Lipocalin Independently Predicts for Renal Response in Myeloma Patients with Severe Renal Impairment**. Presented in: 28th Annual meeting of the Hellenic Society of Haematology (2017), Athens, Greece, 2-4 November 2017.

- Nikolaos Kanellias, Gerasimos-Petros Papassotiriou, Efstathios Kastritis, Emilia Mantzou, Dimitrios Christoulas, Ioannis Ntanasis-Stathopoulos, Anna Komitopoulou, Evangelos Eleutherakis-Papaiakovou, Dimitrios Ziogas, **Despina Fotiou,** Magdalini Migkou, Maria Gavriatopoulou, Ioannis Panagiotidis, Ioannis Papassotiriou, Meletios A. Dimopoulos, Evangelos Terpos. **Circulating Adiponectin and Markers of Endothelial and Cardiovascular Dysfunction Correlate with Disease Burden in Newly Diagnosed Patients with Multiple Myeloma; Increase of Adiponectin after Bortezomib- and IMiD-Based Regimens**. Presented in: 28th Annual meeting of the Hellenic Society of Haematology (2017), Athens, Greece, 2-4 November 2017.

- E.Kastritis, M.Gavriatopoulou, M.Roussou, T.Bagratuni, M.Migkou**, D.Fotiou**, D.Ziogas, I. Ntanasis-Stathopoulos, I.Panagiotidis, N.Kanellias, E.Psimenou, A.Papathoma, E.Gakiopoulou, S.Marinaki, I.Papadopoulou, K.Pampoucas, A.Ntalianis, A.Tasidou, E.Terpos, M.A.Dimopoulos. **Salvage Therapy with Lenalidomide in patients with AL Amyloidosis**. Presented in: 28th Annual meeting of the Hellenic Society of Haematology (2017), Athens, Greece, 2-4 November 2017.

- Despina Fotiou, Theodoros N. Sergentanis, Loula Papageorgiou, Kimon Stamatelopoulos, Maria Gavriatopoulou[1], Efstathios Kastritis[1], Theodora Psaltopoulou[1], Stella Salta, Patrick Van Dreden, Rabiatou Sangare, Annette K Larsen, Evangelos Terpos, Ismail Elalamy, Meletios A Dimopoulos, Grigoris T Gerotziafas. In Newly Diagnosed Multiple Myeloma Patients, Longer Procoagulant Phospholipid-Dependent Clotting Time, Higher Levels of P-Selectin, D-Dimers and Thrombin Generation Peak Are Associated with Increased Risk of Resistance to Treatment: Results of the Prospective Roadmap-MM Study**.** Presented in: 27th Annual meeting of the Hellenic Society of Haematology (2017), Athens, Greece, 2-4 November 2017.

Member of scientific committees

Athens Medical Association:
Membership number: 077934, registration since 2013

General Medical Council UK
Reference number: 7281146, Registration since: 2013

Hellenic Society of Hematology (member since 2015)

European Hematology Association (member since 2014)

Hellenic Society for the Study of Cancer Biology; founding member (2018)

Tutorships - Teaching

Msc Clinical Trials: Design and Conduct. National and Kapodistrian University of Athens:	2017-2018 and 2018-2019
MRCP Peer-Peer Revision Group Roles: Organising the group, Teaching Brighton Sussex University Hospitals	2/2013-08/2013
Regional teaching program for medical students Lectures covering: Renal and Elderly Medicine & Gynaecology for Finals Brighton Sussex University Hospitals	2012-2013
4th year Medical Student Supervisor Cambridge University, UK	2011-2012
Social Care program, Municipality of Kifisia Tutoring children with learning disabilities, Athens, Greece	09/ 2003 – 05/ 2004

Seminars - Courses

April 2017	**ALS Provider Course, European Resuscitation Council** Hellenic Society of Cardiopulmonary Resuscitation Athens, Greece

April 2015 Good Clinical Practice
NIADI GCP Learning Center National Institute of Allergy and Infectious Diseases

Honors and Awards

- **Despina Fotiou**, Theodoros N. Sergentanis, Loula Papageorgiou , Maria Gavriatopoulou, Theodora Psaltopoulou, Patrick VanDreden, Annette K Larsen, Ismail Elalamy Efstathios Kastritis, Kimon Stamatelopoulos, Evangelos Terpos, Meletios A. Dimopoulos, Grigoris T. Gerotziafas. **Prospective assessment of biomarkers of hypercoagulability for the identification of newly diagnosed chemotherapy naïve patients with multiple myeloma at risk for cancer-associated thrombosis. ROADMAT-CAT-MM STUDY.** Presented in: EMLTD 26th Anniversary International Congress on Thrombosis. 19-22 June 2019. Athens, Greece. Best poster presentation award.

- Ioannis V. Kostopoulos, Efstathios Kastritis, P. Micheli, **Ioannis Ntanasis-Stathopoulos**, Magdalini Migkou, Nikolaos Kanellias, **Despina Fotiou,** Evangelos Eleutherakis-Papaiakovou, Maria Gavriatopoulou, Aristea-Maria Papanota, Marilyn Spyropoulou-Vlachou, Ioannis P. Trougakos, Ourania E. Tsitsilonis, Bruno Paiva, Meletios A. Dimopoulos, Evangelos Terpos. Impact of Minimal Residual Disease Detection by Next-Generation Flow 4 Cytometry in Multiple Myeloma Patients with Sustained Complete Remission after Frontline Therapy.
Presented in: 29th Annual meeting of the Hellenic Society of Haematology (2018), Thessaloniki, Greece, 1-4 November 2018. – “Phaedon Phessas” award

Languages

- **Greek** (native)
- **English**- (Proficiency in English, University of Michigan)
- **German**- (Mittelstufe, Goethe Institut)

Extracurricular activities

- IT skills: SPSS, Microsoft Office (Word, Excel, Powerpoint), Google sheets, Pubmed, Powerlab 8/30, Lab Chart 6.0 Pro

- Music: Flute in an Advanced level (1994-2005) and Theory of Music (1994-2005)