[2013]

UNIVERSITY OF ATHENS

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# [ THE PROGNOSTIC VALUE OF BRAF AND NRAS GENES IN MELANOMA PATIENTS

# -

# Η ΠΡΟΓΝΩΣΤΙΚΗ ΑΞΙΑ ΤΩΝ ΓΟΝΙΔΙΩΝ BRAF ΚΑΙ NRAS ΣΕ ΑΣΘΕΝΕΙΣ ΜΕ ΜΕΛΑΝΩΜΑ ]

# *Abstract*

**[Background:** Melanoma is an aggressive cancer with an increasing incidence. Despite many clinical trials, improvement in overall survival in metastatic melanoma remains a significant challenge. Lately, oncogenes have been found in melanoma, as well as in other cancers which may play a role on either oncogenesis or progression to metastases. BRAF and NRAS are the two most common found in melanoma.

**Methods:** We performed systematic computerized searches of the MEDLINE and other available databases to identify all published articles reporting on *NRAS* and *BRAF* somatic mutation analysis in melanoma and included all studies with reported or extractable Hazard Ratios (HRs) from either Individual Patient Data (IPD) or Kaplan-Meier (KM) survival plots.

**Statistical analysis:** We used the *HR* and corresponding CI extracted from each study to assess between-study heterogeneity using the Q statistic and inconsistency using the $I^2$ statistic. Summary *HRs* with their 95% CI were calculated using an inverse variance method. We fitted both random and fixed-effects models and also did an IPD meta-analysis. For the meta-analysis, OS, DFS and MSS were defined as the primary outcomes and subgroup analyses as secondary outcomes. We assessed whether there was a differential magnitude of effects in large versus small studies, commonly referred to as "publication bias", using the Egger and Peter regression tests. We also checked for publication bias using funnel plots, radial plots and funnel plots with the trim and fill method.

**Results:** We found an increased risk of death by 33% (p=0.054) in the random-effects and 27% in the fixed-effects (p=0.0058) analysis for BRAF mutated tumours compared to their wild-type counterparts. The risk of death when the time starts from the time of metastatic diagnosis is 44% for BRAF mutated cases (p=0.0298). No statistical significance was found for BRAF and DFS and none was found for any outcome for NRAS.

**Conclusion:** The BRAF mutation is a negative prognostic factor of the overall survival in melanoma cases. However, this has proven statistical significant only for stage IV metastatic melanoma cases. Further research is needed to identify the prognostic value of the BRAF gene in disease specific survival of all stages of melanoma.**]**

# *Abstract in Greek*

**[Ιστορικό:** Το μελάνωμα είναι ένας επιθετικός καρκίνος με αυξανόμενη συχνότητα. Παρά τις πολλές κλινικές δοκιμές η αύξηση του προσδόκιμου επιβίωσης στο μεταστατικό μελάνωμα παραμένει απογοητευτική. Τελευταία, στο μελάνωμα και σε άλλους καρκίνους έχουν βρεθεί ογκογονίδια που μπορεί να παίζουν ρόλο στην ογκογένεση ή στη μετάβαση σε μεταστατικό μελάνωμα. Τα γονίδια BRAF και NRAS είναι τα δύο πιο συνηθισμένα στο μελάνωμα.

**Μέθοδοι:** Διενεργήσαμε συστηματική έρευνα με υπολογιστή της βάσης δεδομένων MEDLINE καθώς και άλλων, ώστε να αναγνωρίσουμε όλα τα δημοσιευμένα άρθρα που αναφέρονται στο BRAF και NRAS στο μελάνωμα και επιλέξαμε τις μελέτες εκείνες που είτε αναφέρουν είτε μπορούν να υπολογιστούν οι λόγοι κινδύνου ( Hazard Ratios) από ατομικά δεδομένα ασθενή (Individual Patient Data) η γραφικές καμπύλες Kaplan-Meier.

**Statistical analysis:** Χρησιμοποιήσαμε τα HRs και τα διαστήματα εμπιστοσύνης από κάθε μελέτη για να μελετήσουμε την ετερογένεια μεταξύ των μελετών χρησιμοποιώντας το στατιστικό Q και το $I^2$. Οι υπολογισμοί των HRs και των 95% διαστημάτων εμπιστοσύνης έγιναν με τη μέθοδο της ανάστροφης διακύμανσης. Χρησιμοποιήσαμε μοντέλα σταθερών και τυχαίων επιδράσεων και επίσης μετα-αναλύσαμε τα ατομικά δεδομένα ασθενών που είχαμε στη διάθεσή μας. Το συνολικό προσδόκιμο επιβίωσης, το ειδικό για μελάνωμα προσδόκιμο επιβίωσης και το προσδόκιμο επιβίωσης χωρίς υποτροπή ή μετάσταση ήταν οι κύριοι δείκτες της μελέτης μας. Η μελέτη υπο-ομάδων αποτέλεσαν τους δευτερεύοντες στόχους της μελέτης μας. Εξετάσαμε για σφάλμα δημοσίευσης χρησιμοποιώντας τα τεστ των Egger και Peters, καθώς και με διαγράμματα φουγάρου, ακτινικά και τη μέθοδο «κόψε και συμπλήρωσε».

**Results:** Βρήκαμε αυξημένο κίνδυνο θανάτου κατά 33% (p=0.054) στο μοντέλο τυχαίων-επιδράσεων και 27% στο μοντέλο σταθερών επιδράσεων (p=0.0058) για ασθενείς με μελάνωμα και μετάλλαξη στο γονίδιο BRAF σε σχέση με αυτούς που δεν είχαν αυτή τη μετάλλαξη. Ο κίνδυνος αυτός είναι 44% όταν το προσδόκιμο επιβίωσης προσμετράται από τη στιγμή της μετάστασης για τους ασθενείς με μετάλλαξη στο BRAF γονίδιο (p=0.0298). Καμία στατιστική σημαντικότητα δεν βρέθηκε για το προσδόκιμο επιβίωσης χωρίς υποτροπή για το γονίδιο BRAF και για οποιοδήποτε δείκτη για το γονίδιο NRAS.

**Conclusion:** Η μετάλλαξη στο γονίδιο BRAF είναι αρνητικός προγνωστικός παράγοντας για το συνολικό προσδόκιμο επιβίωσης των ασθενών με μελάνωμα. Ωστόσο, αυτό είναι στατιστικά σημαντικό μόνο για τους ασθενείς με στάδιο IV μεταστατικό μελάνωμα. Περισσότερη έρευνα χρειάζεται για να αποδείξει την προγνωστική αξία του γονιδίου σε όλα τα στάδια του μελανώματος.**]**

**ΜΠΣ ΒΙΟΣΤΑΤΙΣΤΙΚΗ**

**ΕΘΝΙΚΟ ΚΑΙ ΚΑΠΟΔΙΣΤΡΙΑΚΟ ΠΑΝΕΠΙΣΤΗΜΙΟ ΑΘΗΝΩΝ**

ΙΑΤΡΙΚΗ ΣΧΟΛΗ

ΤΜΗΜΑ ΜΑΘΗΜΑΤΙΚΩΝ

ΠΑΝΕΠΙΣΤΗΜΙΟ ΙΩΑΝΝΙΝΩΝ

ΤΜΗΜΑ ΜΑΘΗΜΑΤΙΚΩΝ

**Δ Ι Π Λ Ω Μ Α Τ Ι Κ Η   Ε Ρ Γ Α Σ Ι Α**

ΠΟΥΛΙΟΣ ΑΡΙΣΤΟΤΕΛΗΣ

# Η ΠΡΟΓΝΩΣΤΙΚΗ ΑΞΙΑ ΤΩΝ ΓΟΝΙΔΙΩΝ BRAF ΚΑΙ NRAS ΣΤΟΥΣ ΑΣΘΕΝΕΙΣ ΜΕ ΜΕΛΑΝΩΜΑ

ΑΘΗΝΑ, ΙΑΝΟΥΑΡΙΟΣ 2013

Η παρούσα διπλωματική εργασία εκπονήθηκε στο πλαίσιο των σπουδών για την απόκτηση του Μεταπτυχιακού Διπλώματος Ειδίκευσης στη

**ΒΙΟΣΤΑΤΙΣΤΙΚΗ**

που απονέμει η Ιατρική Σχολή και το Τμήμα Μαθηματικών του Εθνικού & Καποδιστριακού Πανεπιστημίου Αθηνών και το Τμήμα Μαθηματικών του Πανεπιστημίου Ιωαννίνων.

Εγκρίθηκε την……………………………………….. από την εξεταστική επιτροπή:

| **ΟΝΟΜΑΤΕΠΩΝΥΜΟ** | **ΒΑΘΜΙΔΑ** | **ΥΠΟΓΡΑΦΗ** |
|---|---|---|
| **Φ. ΣΙΑΝΝΗΣ (Επιβλέπων)** | **ΕΠ. ΚΑΘΗΓΗΤΗΣ** | **………………** |
| **Χ. ΜΠΑΜΙΑ** | **ΕΠ. ΚΑΘΗΓΗΤΡΙΑ** | **………………..** |
| **S. MURRAY** | **PhD, Εξ. Ειδικός** | **……………….** |

## *ΑΦΙΕΡΩΣΕΙΣ*

*Στη γυναίκα μου Μαίρη*
*και τα παιδιά μου Χριστόφορο και Λίλλυ*
*για όλες τις ώρες που έμεινα μακριά τους*
*προωθώντας την έρευνα.*

# *ΕΥΧΑΡΙΣΤΙΕΣ*

*Ευχαριστώ την τριμελή μου επιτροπή*

*Φώτη Σιάννη*
*Sam Murray*
*και Χριστίνα Μπάμια*

*για την αμέριστη υποστήριξή τους στη δύσκολη αυτή εργασία.*

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## *Introduction*

Meta-analysis is one of the hottest topics in Biostatistics within the last few years and new methods are developed, almost every year. On the other hand, gene targeted therapy is one of the hottest topics in Medicine within the last few years with new medications coming into the open market. In this thesis, we try to combine both, by investigating through a systematic review and meta-analysis the prognostic value of BRAF and NRAS genes to patients with melanoma. Recently, new medications have been developed that claim to extend the survival of patients with metastatic melanoma with a BRAF mutated gene. But are their claims founded and is it true that BRAF mutated melanoma patients have worse prognosis than the non-mutated? The evidence is scarce at best and contradictory. With our work we try to shed light into this question and either support or "blow the whistle" to the pharmaceutical companies and the medical community.

As not all of our readers will be familiar with either the melanoma cancer or the meta-analysis techniques we start be describing, in a few words, the basics of both. We then go on to describe our methodology and analysis based on the theoretical knowledge that we have developed previously. Finally we summarize our results and discuss their interpretation in view of the so far known evidence in the medical literature. We hope that you will enjoy the reading!

## *Melanoma*

Melanoma is a malignant tumour of the skin that is known to have the worst prognosis of all the cancers of the skin and remains the leading cause of death from skin disease. The word comes from the Greek word “μέλας”, pronounced melas, meaning dark. It causes about 75% of the deaths that are related to skin cancer (Jerant et al., 2000), although is less common than other skin cancers like basal cell or squamous cell carcinoma. The incidence of cutaneous melanoma is increasing throughout most of the western world (Parkin et al., 2005).

It is caused by changes in cells called melanocytes, which produce a skin pigment called melanin. Melanin is responsible for skin and hair colour. Melanoma can also appear in mucosal surfaces, like the oral cavity, the bowel or even the eye. The overall lifetime risk of getting melanoma is about 2% (1 in 50) for whites, 0.5% (1 in 200) for Hispanics and 0.1% (1 in 1000) for blacks (American_Cancer_Society, 2012). From that is shown that the primary cause for melanoma is the ultraviolet light exposure interacting with the amount of skin pigmentation in the population. Predisposing gene mutations play also their role in the pathogenesis of melanoma. It is probably more of a multistep process that may include the phases of benign naevus, dysplastic naevus, in-situ melanoma, radial and vertical growth phase melanoma and metastatic melanoma (Clark et al., 1984).

Melanoma is comprised of biologically distinct subtypes. The initial World Health Organization (WHO) classification of skin tumours distinguished four main subtypes of melanomas: superficial spreading melanoma (SSM), lentigo maligna melanoma (LMM), nodular melanoma (NM), and acral lentiginous melanoma (ALM) (Clark and Mihm, 1969). Now, there are a lot more:

- Lentigo maligna
- Lentigo maligna melanoma

- Superficial spreading melanoma
- Acral lentiginous melanoma
- Mucosal melanoma
- Nodular melanoma
- Polypoid melanoma
- Desmoplastic melanoma
- Amelanotic melanoma
- Soft-tissue melanoma
- Melanoma with small nevus-like cells
- Melanoma with features of a Spitz nevus
- Uveal melanoma

However, the most common ones are superficial spreading, nodular, lentigo maligna melanoma and acral lentiginous. Superficial spreading melanoma is the most common type, most common in Caucasians and usually flat and irregular in shape and colour, with different shades of black and brown. Nodular melanoma usually starts as a raised area that is dark blackish-blue or bluish-red. Lentigo maligna melanoma usually occurs in the elderly, in sun-damaged skin on the face, neck, and arms. The abnormal skin areas are usually large, flat, and tan with areas of brown. Finally, acral lentiginous melanoma is the least common form. It usually occurs on the palms, soles, or under the nails, is more common in African Americans and does not seem to be associated with ultraviolet light exposure. The risk of developing melanoma increases with age, although it can also be seen in young people and children. Melanoma is not as common as other types of skin cancer.

Risk factors for developing melanoma are fair skin, blue or green eyes, red or blond hair, living in sunny climates or at high altitudes, spending a lot of time in high levels of strong sunlight, because of job or other activity, having had one or more blistering sunburns during childhood or using tanning devices. Other risk factors include close relatives with a history of melanoma, atypical or dysplastic

moles or multiple birthmarks or weakened immune system due to disease or medication.

There are two histopathologic grading systems for melanoma tumours, the Clark, which is more anatomical and the Breslow, which assesses the depth of the tumour (Breslow, 1970; Clark, 1976). The Clark system has a lower predictive value, is less reproducible, is more operator-dependent and is useful only when the Breslow thickness is less than 1mm. Both systems can be seen below:

**Clark's grading system**

| | |
|---|---|
| Level 1 | Melanoma confined to the epidermis (melanoma in situ) |
| Level 2 | Invasion into the papillary dermis |
| Level 3 | Invasion to the junction of the papillary and reticular dermis |
| Level 4 | Invasion into the reticular dermis |
| Level 5 | Invasion into the subcutaneous fat |

**Breslow's thickness**

| | |
|---|---|
| Stage I | less or equal to 0.75mm |
| Stage II | 0.75 mm - 1.5mm |
| Stage III | 1.51 mm - 2.25mm |
| Stage IV | 2.25 mm - 3.0mm |
| Stage V | greater than 3.0 mm |

Currently, the most powerful prognostic factors for cutaneous melanoma at early stage are Breslow's tumour thickness, ulceration of the primary tumour, and sentinel lymph node status. The Breslow's thickness has been incorporated into the clinical grading system defined by the American Joint Committee on Cancer (Balch et al., 2009) and can be seen below:

**Melanoma stages with 5 year survival rates:**

| | | |
|---|---|---|
| **Stage 0:** | Melanoma in situ (Clark Level I), 99.9% survival | |
| | | |
| **Stage I:** | Invasive melanoma, 85–99% survival | |
| | **T1a:** | Less than 1.00 mm primary tumor thickness, without ulceration, and mitosis < $1/mm^2$ |
| | **T1b:** | Less than 1.00 mm primary tumor thickness, with ulceration or mitoses ≥ $1/mm^2$ |
| | **T2a:** | 1.00–2.00 mm primary tumor thickness, without ulceration |
| | | |
| **Stage II:** | High risk melanoma, 40–85% survival | |
| | | |
| | **T2b:** | 1.00–2.00 mm primary tumor thickness, with ulceration |
| | **T3a:** | 2.00–4.00 mm primary tumor thickness, without ulceration |
| | **T3b:** | 2.00–4.00 mm primary tumor thickness, with ulceration |
| | **T4a:** | 4.00 mm or greater primary tumor thickness without ulceration |
| | **T4b:** | 4.00 mm or greater primary tumor thickness with ulceration |
| | | |
| **Stage III:** | Regional metastasis, 25–60% survival | |
| | **N1:** | Single positive lymph node |
| | **N2:** | Two to three positive lymph nodes or regional skin/in-transit metastasis |
| | **N3:** | Four positive lymph nodes or one lymph node and regional skin/in-transit metastases |
| | | |
| **Stage IV:** | Distant metastasis, 9–15% survival | |
| | **M1a:** | Distant skin metastasis, normal LDH |
| | **M1b:** | Lung metastasis, normal LDH |
| | **M1c:** | Other distant metastasis or any distant metastasis with |

| | | elevated LDH |
|---|---|---|

The treatment of early stage melanoma is surgical excision of the lesion with adequate margins. Although early melanomas are often cured by surgery, up to 20% of those patients develop metastatic disease (Balch et al., 2009). The probability of recurrence may be defined as low risk, intermediate risk or high risk based on the thickness of the primary tumour, the presence of ulceration or mitoses in the primary tumour and the presence of nodal, in-transit or satellite metastasis. For relapses, intermediate and high risk, or high grade metastatic melanomas, other therapies include chemotherapy (medicines to kill cancer cells), immunotherapy (medications such as interferon or interleukin to help the immune system to fight the cancer) and radiotherapy. Whilst the majority of patients with early stage disease are cured with surgery alone, treatment for metastatic disease is by far lucking success. The response rates to chemotherapy, of patients with metastatic melanoma are of less than 20% and their median survival of less than 12 months (Tsao et al., 2004). Among intermediate stage melanoma patients, there may be considerable differences in patient survival and disease free interval. The molecular findings may be able to help the histopathologic grading in improving our prognosis for the various subgroups of melanoma patients. Despite many clinical trials, improvement in overall survival in metastatic melanoma remains a significant challenge (Crosby et al., 2000). The 5-year survival rate for patients with advanced disease is only 15% (Garbe and Eigentler, 2007; Villanueva and Herlyn, 2008).

## BRAF and NRAS

BRAF is a human gene that makes a protein called B-Raf. The gene is also referred to as proto-oncogene B-Raf or v-Raf murine sarcoma viral oncogene homolog B1, while the protein is more formally known as serine/threonine-protein

kinase B-Raf. About 60% of melanomas contain a mutation in the BRAF gene (Brose et al., 2002; Davies et al., 2002; Goydos et al., 2005).

NRAS is another gene that was discovered by researchers at the Institute of Cancer Research (UK), and named NRAS (Neuroblastoma-RAS viral oncogene homolog), for its initial identification in human neuroblastoma cells (Marshall et al., 1982; Hall et al., 1983). The family of RAS genes consists of three functional oncogenes, H-ras, K-ras, and N-ras, which encode highly similar proteins with molecular weights of Mr 21,000. Activated Ras proteins have been shown to interact with numerous downstream targets, to activate several different signaling pathways, and subsequently become inactivated. Mutated Ras proteins, however, have lost the ability to become inactivated and thus stimulate cellular growth and differentiation continuously (Demunter et al., 2001). Up to 30% of melanoma tumours may contain NRAS mutated genes usually in exon 1 (codon 12) and exon 2 (codon 61) (Lee et al., 2011). The majority of activating BRAF and NRAS mutations in melanomas have been mutually exclusive apart from very few cases (Akslen et al., 2005; Goel et al., 2006).

Both genes affect the MAPK pathway. The mitogen-activated protein kinase pathway (MAPK) is a membrane-to-nucleus signalling system that controls cell proliferation, differentiation, and apoptosis in mammalian cells (Peyssonnaux and Eychene, 2001). This pathway can be activated either due to oncogenic mutations in BRAF and NRAS genes (Peyssonnaux and Eychene, 2001) or through autocrine growth factor stimulation (Satyamoorthy et al., 2003). Involving proteins are RAS, RAF, MEK and ERK. Activated RAS proteins are membrane-bound small G proteins, whereas RAF, MEK, and ERK are cytosolic protein kinases that form a tiered protein kinase cascade downstream of RAS (Ugurel et al., 2007). NRAS can also up-regulate the Phosphatidylinositor-3-Kinase (PI3K) and RAL pathways resulting in inhibition of apoptosis, cell proliferation, invasion and anchorage independent growth (Mishra et al., 2010). There are three RAF proteins in mammals, A-RAF, B-RAF, and C-RAF, which can all activate MEK. A-

RAF and C-RAF have not been found to be mutated because their regulation is fundamentally different from that of B-RAF. The BRAF mutant possesses a tenfold greater basal kinase activity and induces focus formation in NIH3T3 cells 138 times more efficiently than does wild-type (Davies et al., 2002), thus having a central role in melanocyte proliferation. Inhibition of BRAF or NRAS could theoretically lead to improvements in the treatment of malignant melanoma. Other genes include tumour suppressor genes (p16 and PTEN), cell adhesion molecules (E-cadherin), and metalloproteinases (mmp-2) (Hsu et al., 2002).

The most common of BRAF mutations is known as V600E and results from substitution of the valine residue at amino-acid position 600 to glutamic acid in the B-raf protein, locking the kinase into an active state (Davies et al., 2002). Oncogenic mutations of this gene have been found also in breast cancer (ERBB2 or HER2), non–small-cell lung cancer (EGFR), colorectal cancer (KRAS), chronic myelogenous leukemia (ABL), and GI stromal tumours (CKIT) (Brose et al., 2002; Davies et al., 2002).

Recently, novel therapies, against activated genes like the BRAF show promising results for the metastatic melanoma.

### *Meta-analysis*

Meta-analysis is the statistical method used to combine the results of related studies and provide a pooled summary effect estimate. It provides a quantitative assessment of an otherwise qualitative assessment that is done through a systematic review.

The volume of the available medical or other literature is constantly expanding, especially with the beginning of the electronic age and the widespread use of computers. In this climate of constantly expanding knowledge, systematic reviews have been one way of summarizing the available evidence in one's field and identifying areas where this is insufficient and needs further research. Systematic review is a type of research that attempts to identify and summarize all of the evidence related to a specific research question (Bent et al., 2004). Systematic reviews should not be confused with traditional narrative reviews. Systematic reviews use systematic, explicit, objective and prospectively-defined methods to identify, select and critically appraise the relevant primary research and to extract and analyse data from the included studies (The_Cochrane_Collaboration, 2005). The statistical process component of the systematic review is the one that called meta-analysis. There are 8 steps to conducting a systematic review (Bent et al., 2004):

1. Formulate the **appropriate research question**.
2. Develop a **protocol**.
3. Initiate a **search strategy** through the available literature, databases and other available data collection points.
4. Identify the **inclusion and exclusion criteria** for the studies to be eligible and selected.
5. Assess the **quality** of the studies to be included.
6. **Extract** the available data from the included studies.

7. **Analyse** the data.
8. **Interpret** the results.

Because meta-analysis uses the evidence from all the included studies, can provide more precise estimates and allows for decisions based on the whole of the available evidence. With a meta-analysis we gain more statistical power, more accurate representation of the population relationship and assess the heterogeneity of the involved studies in the process. In order for meta-analysis to be free from bias, it should be based on randomised controlled trials, but meta-analysis of prospective cohort studies or diagnostic studies is also possible. We should note here that meta-analysis is the statistical procedure of combining the available evidence and it is not a different type of review. The Cochrane Collaboration has been instrumental in organizing the necessary framework and providing many of the tools the researchers need for a proper systematic review and meta-analysis (Chalmers, 1993). The Cochrane Collaboration is named in honour or Archie Cochrane, a British research, who first realised the need for an organised critical summary, by specialty or subspecialty, adapted periodically, of all relevant randomized controlled studies.

Meta-analysis can also help in the designing of new studies. It can define the expected effect size from previous evidence, and hence guide to power calculations and sample size estimation. It can also identify the sources of heterogeneity of the previous studies by the process of meta-regression and suggest the effect estimates in different subgroups (e.g. males, females e.t.c.)(Braithwaite, 2009).

Meta-analysis can be based on aggregate data, which are extracted from the reports of the published studies or individual patient assessment data (IPD), which are either extracted from the published studies or collected from the authors of those studies. Although IPD meta-analysis is the gold standard, most

of the time is not possible as the authors of most of the studies have to be contacted and agree to share their data.

Meta-analysis usually follows a 2-stage approach (Tinazzi and Tierney, 2007). In the first stage, summary statistics are generated for each trial. In the second stage, a final estimate is summarized (pooled) from the primary summary statistics. Additionally, a weight is assigned to each study before the calculation of the pooled effect. The formula used is the following:

$$\text{pooled effect estimate} = \frac{\sum(study\ effect\ estimate * weight)}{\sum(weight)}$$

For dichotomous outcomes, relative risk (RR), odds ratio (OR) and risk difference (RD) can be calculated. For continuous outcomes the results can be expressed as mean with standard deviation (SD), difference in means (MD) or standardised mean difference (SMD). For time-to-event outcomes the usual measure it the hazard ratio (HR). The HR for an i study has the following form:

$$HR_i = exp\left(\frac{\sum O_i - E_i}{\sum V_i}\right)$$

Where $O_i$ is the observed number of events in the research intervention and $E_i$ is the expected number of events in the research intervention under the null hypothesis of equality among treatment intervention and control and $V_i$ the hyper-geometric variance. An HR greater than 1 indicates that the condition or event is more likely in the research group and the opposite for values less than 1.

## Fixed and Random effects meta-analysis

In combining the study results, two models can be used: the fixed effects and the random effects model (Borenstein et al., 2009).

Under the fixed-effect model we assume that there is one true effect size (hence the term fixed effect) which underlies all the studies in the analysis, and that all differences in observed effects are due to sampling error. By contrast, under the random-effects model we allow that the true effect could vary from study to study. Because the studies will differ in their mix of various characteristics, there may be different true effects underlying different studies. In this case, there are two sources of expected variation. One is the within study sampling error which we know from the fixed effects. The other is the between-study variation due to the different "true" or population effect estimate for each study. The between-study variability is symbolized with $\tau^2$.

A study's true effect size is the effect size in the underlying population. In other words, it is the effect we would observe if we were able to do the study on the whole of the population in question. A study's observed effect is the one reported by the study and is subjected to sampling and other statistical errors.

In a fixed effect model the observed effect $Y_i$ for any study $i$, is given by the population mean (or true effect) $\theta$ plus the sampling error $\varepsilon_i$ in that study as follows:

$$Y_i = \theta + \varepsilon_i$$

The weight assigned to each study *i* in a fixed-effect meta-analysis is:

$$W_i = \frac{1}{V_i}$$

where $V_i$ is the within-study variance of that study.
The weighted treatment effect is then computed by the following formula:

$$Pooled\ effect\ under\ fixed\ effects\ model = \frac{\sum_{i=1}^{k} W_i Y_i}{\sum_{i=1}^{k} W_i}$$

which is the sum of the products $W_i Y_i$ (effect size for each study multiplied by each weight) divided by the sum of weights. The variance of the pooled effect is estimated by:

$$V_{pooled\ effect} = \frac{1}{\sum_{i=1}^{k} W_i}$$

and the standard error by:

$$SE_{pooled\ effect} = \sqrt{V_{pooled\ effect}}$$

The 95% lower and upper limits of the pooled effect are estimated as:

$$LL_{pooled\ effect} = pooled\ effect - 1.96\ x\ SE_{pooled\ effect}$$

$$UL_{pooled\ effect} = pooled\ effect - 1.96\ x\ SE_{pooled\ effect}$$

The Z-value, to test for the null hypothesis that the common true population effect $\theta$ is zero is computed by:

$$z = \frac{pooled\ effect}{SE_{pooled\ effect}}$$

For a fixed effects model the summary points as they are pointed out by Borenstein et al., 2009 are:

- All studies in this model share a common true population effect.

- The pooled effect is OUR estimate of this common effect size, and the null hypothesis is that this common effect is zero (for a difference) or one (for a ratio).
- All the observed dispersion among the various studies estimates reflects only sampling error, and the study weights are assigned with the goal of minimizing this within-study error.

In a random-effects model the observed effect $Y_i$ for any study $i$, is given by the grand population mean (or grand true effect) $\mu$ plus the deviation of the study's true effect $\theta_i$ from the grand mean $\mu$ plus the sampling error $\varepsilon_i$ in that study from its true effect $\theta_i$ as follows:

$$Y_i = \theta_i + \varepsilon_i = \mu + \zeta_i + \varepsilon_i$$

It is obvious that we know have many true effects $\theta_i$ rather than one as in the fixed-effects model. Therefore, to predict how far the observed effect $Y_i$ is likely to fall from $\mu$ in any given study we need to know the variance of $\zeta_i$ as well as the variance $\varepsilon_i$ . This standard deviation of the distribution of the true effects of the included studies is called $\tau$ (tau) or $\tau^2$ for its variance. In other words, this is the between-study variance. The same value of $\tau^2$ applies to all studies in the meta-analysis.

One method of estimating $\tau^2$ is the method of moments or DerSimonian and Laird (DerSimonian and Laird, 1986) as below:

$$\tau^2 = \frac{Q - df}{C}$$

where

$$Q = \sum_{i=1}^{k} W_i Y_i^2 - \frac{\left(\sum_{i=1}^{k} W_i Y_i\right)^2}{\sum_{i=1}^{k} W_i}$$

with *df=k-1*, where *k* is the number of *i* studies, and

$$C = \sum W_i - \frac{\sum W_i^2}{\sum W_i}$$

This method does not make any assumptions about the distribution of the random effects. It is the easiest to compute and explain but some researchers prefer the restricted maximum likelihood (REML) method. However, the differences in results from one method to the other are likely to be small. For both fixed and random effects models we follow the inverse-variance method, in which each study is weighted by the inverse of its variance. In the random-effects model the variance includes both the between and the within-study variance. Therefore, the weight assigned to each study *i* in a random-effects meta-analysis is:

$$W_i^* = \frac{1}{V_{Y_i}^*}$$

where $V_{Y_i}^*$ is the within-study variance of that study i plus the between-studies variance $\tau^2$ as:

$$V_{Y_i}^* = V_{Y_i} + \tau^2$$

The weighted treatment effect is then computed by the following formula:

$$pooled\ effect\ under\ random - effects\ model = pooled\ effect^* = \frac{\sum_{i=1}^{k} W_i^* Y_i}{\sum_{i=1}^{k} W_i^*}$$

which is the sum of the products $W_i^* Y_i$ (effect size for each study multiplied by each weight) divided by the sum of weights. The variance of the pooled effect is estimated by:

$$V_{pooled\ effect\ under\ random-effects\ model} = \frac{1}{\sum_{i=1}^{k} W_i^*}$$

and the standard error by:

$$SE_{pooled\ effect^*} = \sqrt{V_{pooled\ effect\ under\ random-effects\ model}}$$

The 95% lower and upper limits of the pooled effect are estimated as:

$$LL_{pooled\ effect^*} = pooled\ effect^* - 1.96\ x\ SE_{pooled\ effect^*}$$

$$UL_{pooled\ effect^*} = pooled\ effect^* - 1.96\ x\ SE_{pooled\ effect^*}$$

The Z-value, to test for the null hypothesis that the grand true population effect $\mu$ is zero is computed by:

$$z^* = \frac{pooled\ effect^*}{SE_{pooled\ effect^*}}$$

For a random-effects model the summary points, again, as they are pointed out by Borenstein et al., 2009 are:

- The true effects in the studies are assumed to have been sampled from a distribution of true effects.
- The pooled effect is OUR estimate of the grand mean of the true study effects, and the null hypothesis is that this grand mean is zero (for a difference) or one (for a ratio).
- In the random-effects model we need to take into account two sources of variance. One is the within-study variance in estimating the effect in each study. The other is the between-studies variation of the true effects across

all the studies. The study weights are assigned in such a way to minimize both sources of variance.

Obviously, the question is which model to choose. According to Borenstein et al. 2009 in most of the cases, the studies will differ between them in various characteristics and in that case a random-effects model would be the most appropriate. In reality there are only very few cases where all the studies are identical and then a fixed-effects model would make sense, i.e. identical repeated trials by a pharmaceutical company. In such cases, a fixed-effects model is more powerful and easier. However, no inference can be made to the population but only to the total of patients that included in the study. The study weights are more balanced under the random-effects model, where large studies are assigned less relative weight than they would have in a fixed-effects model. Also, the standard error of the pooled effect and its confidence intervals will be wider under the random-effects model, since the variability increases by taking two sources of variance into account.

The problem arises when the number of studies is small and therefore not enough to calculate a precise estimate of the between-studies variance ($\tau^2$). In that case, Borenstein et al., 2009 suggest to either report the separate effects and not a pooled effect or to perform a random-effect meta-analysis or a Bayesian meta-analysis. In the later, the between-studies variance is estimated from data outside the current set of studies. Although the last one is considered the best option, especially if we want to generalise our findings to the whole population, what is usually preferred is a random-effect meta-analysis. In any case the decision should be taken from the beginning of the study and not based on the tests for heterogeneity which have low power and sensitivity when the number of studies is small. We should note here that the results of a fixed-effects meta-analysis are still valid mathematically. However, the inference can be made only for the population of the studies and not the general population and under the strong assumption that all the studies measure the same true effect.

## Heterogeneity

Heterogeneity is the result of the different characteristics of each combined study. The studies differ in the composition of patients, in their design, interventions, outcomes, e.t.c. It can be subcategorized in clinical heterogeneity and statistical heterogeneity. Clinical heterogeneity may be defined as the presence of variation in true effect sizes underlying the different studies in magnitude or direction (Higgins, 2008). Statistical heterogeneity may be caused by clinical, methodological or unknown characteristics between the studies. We should note here that even clinically homogenous study may be heterogeneous statistically. Also the heterogeneity that we may observe, incorporates both true heterogeneity and sampling error.

The p-value for Cochran's Q is the test statistic which is usually used to describe whether the results of each study are really heterogeneous or their variability is due to sampling error. It does not measure heterogeneity. The test looks at the differences between observed effects of the studies and the pooled effect estimate. It tests the null hypothesis that the studies all have the same effect in the population. It is calculated by the following formula for *i*=*k* studies:

$$Q = \sum_{i=1}^{k} W_i (Y_i - M)^2 = \sum_{i=1}^{k} W_i Y_i^2 - \frac{\left(\sum_{i=1}^{k} W_i Y_i\right)^2}{\sum_{i=1}^{k} W_i} = \sum_{i=1}^{k} \left(\frac{Y_i - M}{S_i}\right)^2$$

Where $W_i$ is the study weight $(\frac{1}{V_i})$, $S_i$ is the standard deviation, $Y_i$ is the study effect size and $M$ is the summary effect size. In other words, we compute the deviation of each effect size from the mean (which is the pooled effect), square it, weight this by the inverse variance for that study, and sum these values over all studies to yield the weighted sum of squares or Q. As a standardised measure it

is not affected by the metric of the effect size index and is simply equal to the degrees of freedom *df=k-1*. *df* is the expected variation, Q is then the total variation and *Q-df* is the excess variation among the studies. We then pose the null hypothesis that all studies share a common effect size and then test this hypothesis. Under the null hypothesis Q will follow a chi-squared distribution with degrees of freedom equal to *k– 1*, so we can report a p -value for any observed value of Q. If the null hypothesis was true then Q should be equal to the degrees of freedom. As a test of significance is sensitive both to the magnitude of the effect, i.e the excess dispersion and the precision with which this effect is estimated, which here is based on the number of studies.

It is a test with low power, especially in a small number of studies and therefore many researchers prefer to put the limit for statistical significance $p<0.1$ rather than the usual $p<0.05$. If significant heterogeneity exists then it may be invalid to pool the results of each study. We can then:

- narratively describe the results
- or investigate the sources of heterogeneity and do separate analyses, subgroup analyses, or meta-regression,
- or account for that heterogeneity in our analysis and use a random-effects model.

Another measure is the variance of the true effect sizes, which is the parameter $\tau^2$. The difference *Q-df* represents the variance of the true effects but in a standardised way. By dividing by a quantity (*C*) which has the effect of putting the measure back into its original metric and also of making it an average, we get $\tau^2$.

$$C = \sum W_i - \frac{\sum W_i^2}{\sum W_i} \quad and \quad \tau^2 = \frac{Q - df}{C}$$

While the actual variance of the true effects $\tau^2$ can never be less than zero, our estimate of this value can be less than zero if, because of sampling error, the observed variance *Q* is less than the expected *df*. In this case, $\tau^2$ is simply set to zero.

Finally, the between-study variance can also be defined as a ratio in terms of $I^2$. That is defined as the percentage of total variation across studies that is due to between-study variation rather than sampling error (Higgins and Thompson, 2002). It is the percentage of excess variance which is not explained by the variation within the studies and essentially an intraclass correlation coefficient. It is calculated as below:

$$I^2 = \left(\frac{Q - df}{Q}\right) * 100\%$$

Another way to write $I^2$ is:

$$I^2 = \left(\frac{\tau^2}{\tau^2 + V_y}\right) * 100\%$$

and therefore to think of it as the ratio of true heterogeneity to total variance. This is not entirely true as there is not a single $V_Y$, since the within-study variances vary from study to study. It is best if $I^2$ is viewed as a measure of inconsistency between the study effects. $I^2$ is not directly affected by the number of studies in the meta-analysis. It is suggested (Higgins et al., 2003) that if:

$I^2$ = 0% then there is no heterogeneity,
$I^2$ = 25% then there is low heterogeneity,
$I^2$ = 50% then there is moderate heterogeneity,
$I^2$ = 75% then there is high heterogeneity.

It should be noted though that these are arbitrary, except for 0 and $I^2$ can never reach 100%. For obvious reasons, values above 90% are very rare and should be looked upon with cautiousness. Finally, we should note that a high value of $I^2$ does not imply that the effects are dispersed over a wide range as they could fall in a narrow range but be estimated precisely, and the opposite.

On comparing $I^2$ with $\tau^2$, $I^2$ reflects only the proportion of variance that is true, whereas $\tau^2$ reflects only the absolute value of this true variance. Also, it would not be meaningful to compare the $\tau^2$ values for two different meta-analyses, because they would be in a different metric, that of their effects. On the other hand, $I^2$ is on a ratio scale of 0 % to 100 %, and it is possible to compare this value between different meta-analyses.

The effort to understand heterogeneity is not only for our analysis but also to interpret the results. A consistent HR study estimate of 1.4 across many studies is different to a HR that ranges from 0.7 to 2.8, being both different in direction and size. If the effect size is consistent across studies, then the implications are different for the population to which it refers, than when it varies.

## Forest plot

The forest plot is the most common graphical presentation of the individual results of each study included in a meta-analysis together with the combined meta-analysis result (The_Cochrane_Collaboration, 2005). It is also known as "confidence intervals plot" and its origin goes back to the 1970s (Freiman et al., 1978). The results of individual studies are shown as squares centred on each study's point estimate. A horizontal line runs through each square to show each study's confidence interval - usually, but not always, a 95% confidence interval. The overall estimate from the meta-analysis and its confidence interval are

shown at the bottom, represented as a diamond. The centre of the diamond represents the pooled point estimate, and its horizontal tips represent the confidence interval. On the left part of the plot we usually find the study labels and on the right the upper and lower limit of the confidence intervals and the p-value. At the bottom the pooled effect with its confidence interval is also presented. The plot allows readers to see the heterogeneity as well as the results of the studies. Cochrane's Q and $I^2$ are usually presented here along with a p-value.

## Assessment of bias

One particular aspect of meta-analysis is whether the included studies are valid. The authors of the Cochrane Handbook consider two dimensions for the validity of a study (Higgins and Altman, 2008). "External validity" is referred to whether a study is asking the appropriate research question. Its assessment depends on the purpose for which the study is to be used and connected with the generalizability or applicability of a study's findings. "Internal validity" refers to whether the study answers the appropriate question in an "appropriate" way, that is in manner free from bias.

Assessments of internal validity are frequently referred to as 'assessments of methodological quality' or 'quality assessment'. However, this term usually suggests an investigation of the extent to which study authors conducted their research to the highest possible standards. It is possible that a study fulfils the highest of quality standards yet it is still full of bias, e.g. no blinding or allocation concealment because that was not possible.

A bias is a systematic error, or deviation from the truth, in results or inferences. It can result in overestimation or underestimation of treatment effects, can be

minimal or significant. It is usually impossible to know to what extent biases have affected the results of a particular study and therefore more appropriate to consider risk of bias. Obviously, if we knew the effect of a certain bias to our summary effect then we would be able to include it in the calculations and incorporate it in a new summary effect.

Bias should also not be confused with imprecision. Bias refers to systematic error, meaning that multiple replications of the same study would reach the wrong answer on average. Imprecision refers to random error, meaning that multiple replications of the same study will produce different effect estimates because of sampling variation even if they would give the right answer on average. For example, the results of smaller studies are subject to greater sampling variation and hence are less precise as reflected by their confidence intervals. In a meta-analysis this affects the weight that every study is allocated and more precise results are obviously given more weight.

Higgins and Altman, 2008 continue to describe six domains that consist the “The Cochrane Collaboration’s tool” for assessing risk of bias. These are:

- **Sequence generation** which refers to the method used to generate the allocation sequence for randomization. It should be a random sequence generation process such as a random number table, a computer random number generator e.t.c.
- **Allocation concealment** which refers to the method used to conceal the allocation sequence in such a way so that the intervention allocations could not have been foreseen in advance of, or during, enrolment. This could be central allocation (including telephone, web-based and pharmacy-controlled randomization), sequentially numbered drug containers of identical appearance or sequentially numbered, opaque, sealed envelopes.
- **Blinding** of participants, personnel and outcome assessors from knowledge of which intervention a participant received. Blinding is

important for objective outcomes in trials where enthusiasm for participation or follow-up may be influenced by group allocation. With this bias we have to check whether no blinding or incomplete blinding was done, and the outcome or outcome measurement is likely to be influenced by lack of blinding.

- **Incomplete outcome data** which refers to missing participants or outcomes. They divide the incomplete outcome data into two categories: Exclusions, which refer to situations in which some participants are omitted from reports of analyses, despite outcome data being available to the trialists. And attrition, which refers to situations in which outcome data are not available. The incomplete outcome data bias refers to checking whether reason for missing outcome data is likely to be related to true outcome, whether it is possible to induce clinically relevant bias in intervention effect estimate, whether "as-treated" analysis was done with substantial departure of the intervention received from that assigned at randomization and finally, whether there was inappropriate application of simple imputation.
- **Selective outcome reporting** or "within-study publication bias". This refers to statistically significant differences between intervention groups are more likely to be reported than non-significant differences. It checks whether pre-specified primary outcomes have been reported, whether are reported using measurements, analysis methods or subsets of the data (e.g. subscales) that were not pre-specified, whether reported primary outcomes were not pre-specified, whether reported incompletely so that they cannot be entered in a meta-analysis or finally, the authors fail to include results for a key outcome that would be expected to have been reported for such a study. It can be one of the most substantial biases affecting results from individual studies (Chan and Altman, 2005).
- **Other bias** which refers to other circumstances, like bias due to early stopping, data design, extreme baseline imbalance e.g. between males

and females, fraudulence, finance etc. It can be different for different studies as it depends on the type of study and the question asked.

The missing data is a significant issue in meta-analysis. Missing data can be categorized into “missing at random” where the missingness is unrelated to the true missing value or the data themselves and “informatively missing” where the fact that an observation is missing is a consequence of the value of the missing observation (Higgins, 2010). In this second case, which is more common, we are talking about bias and needs careful consideration. There could be either studies that are not reported, usually with negative or non-statistically significant results (publication bias), or in the same study many outcomes may not be reported (selective outcome reporting bias) or data could be missing (incomplete data bias). Sometimes, study characteristics could also be missing making the assessment of heterogeneity more difficult.

A common classification scheme for bias includes selection bias (refers to sequence generation and allocation concealment), performance bias (refers to blinding and other risks), attrition bias (refers to incomplete outcome data and blinding), detection bias (blinding and other threats to validity) and reporting bias (refers to selective outcome reporting).

For all potential sources of bias, it is important to consider the likely magnitude and the likely direction of the bias. For example, if all methodological limitations of studies were expected to bias the results towards a lack of effect, and the evidence indicates that the intervention is effective, then it may be concluded that the intervention is effective even in the presence of these potential biases.

The use of scales for assessing quality or risk of bias is explicitly discouraged by Higgins and Altman because it is not supported by empirical evidence (Emerson et al., 1990; Schulz et al., 1995) and have been shown to be unreliable assessments of validity (Juni et al., 1999). While the approach offers appealing

simplicity, calculating a summary effect inevitably involves assigning 'weights' to different studies in the scale, and it is difficult to justify the weights assigned. It is already said that "perhaps the most insidious form of subjectivity masquerading as objectivity is "quality scoring" (Greenland, 1994)".

The risk of bias in a meta-analysis can have significant effect if one considers that even a very large study at high risk of bias has minimum variance and therefore an increased weight in the meta-analysis. This can also be exaggerated if the meta-analysis includes many large studies with significant bias. Obviously, studies at high or unclear risk of bias should be given reduced weight in meta-analyses, compared with studies at low risk of bias (Spiegelhalter and Best, 2003). However, the major approach to incorporating risk of bias assessments is to restrict meta-analyses to studies at low (or lower) risk of bias. Unfortunately, this is not always possible, so, in general, 3 strategies are suggested:

- As already said, restrict the analysis to studies at low (or low and unclear) risk of bias. After a restricted primary analysis, review authors are encouraged to perform and present sensitivity analyses showing how conclusions might be affected.
- Present all studies and provide a narrative discussion of risk of bias.
- Finally, present multiple analyses. Care should be taken so as to not confuse the readers and also include a simple summary table with the reported outcomes at the end.

## Publication bias

Publication bias describes the tendency for studies reporting uninteresting, unfavourable or non-significant results to be less likely to be published. Meta-analysis of published papers is likely to be affected by publication bias. That is because research is more likely to be published if it has interesting, favourable or

statistically significant results. It has been found that statistically significant results are three times more likely to be published than papers affirming a null result (Dickersin et al., 1987). Positive results bias, a type of publication bias, occurs when authors are more likely to submit, or editors accept, positive than null (negative or inconclusive) results (Sackett, 1979). Negative results bias or "the file drawer problem", refers to the tendency for negative or inconclusive results to remain unpublished by their authors. Unfortunately, well designed research, with large sample sizes, is less likely to produce interesting or favourable results when there is none such true effect. Therefore, combining all the studies leads inevitably in a biased or pooled summary effect estimate.

## Funnel plot

A funnel plot is a graphical presentation that was suggested in order to investigate for publication bias. It is essentially a scatter plot of study effect size (usually on the x-axis) against a measure of study precision on the y-axis. The standard error of the effect estimate is often chosen as the measure of study size and plotted on the vertical axis with a reversed scale that places the larger, most powerful studies towards the top. When no publication bias is present, the funnel plot should resemble an inverted funnel with larger variation in effect sizes being observed in the less precise studies due to sampling error. When small-study effects are present, the funnel will look asymmetrical with a tendency for effect sizes to be larger in the less precise studies, suggesting a missing 'chunk' out of the funnel. This is usually seen on the left hand side since studies with negative effects (assuming that these are not beneficial) will have less chance of being published. Unfortunately, publication bias is not always the cause of a funnel plot asymmetry.

Any factor associated with both study effect and study size could confound the true association and cause an asymmetric funnel plot. Trials of lower quality yield exaggerated estimates of treatment effects, as they are on average, conducted and analysed with less methodological rigor than larger studies (Egger et al., 2003). So asymmetry may also result from the overestimation of treatment effects in smaller studies of lower methodological quality. It has been shown that studies with smaller sizes are more likely to find statistically significant results, when there is none and because of that to get published. This could lead to an exaggerated pooled summary effect with a subsequent asymmetry in the funnel plot because studies with large size and hence smaller bias would concentrate on the top and more narrow part of the funnel plot and the pooled estimate will be pulled by the biased studies.

An interesting feature that sometimes is seen in a funnel plot is the "tunnel" effect (Peters et al., 2008). On visual inspection of such a funnel plot, most of the studies are situated on the left and right of the central line of the funnel plot, meaning that they show significant effect sizes on either direction. On the other hand, few studies exist on the non-significant central core of the funnel plot. That means that studies with statistically significant results in either direction are published when others with non-statistically significant results are suppressed. Although this could be due to sampling error, the ''tunnel'' effect could also arise, when there is selective reporting outcome, meaning that the marginally statistically significant result was selected from a number of possible outcomes or analyses that remain unpublished. Another reason could be that the data were analysed in such a way that finally a statistically significant result was obtained. Because the funnel plot is still symmetric, we still would not expect a biased pooled summary estimate, i.e. a significant result due to publication bias when in fact there is not. However, this also has implications for estimates of between-study variance (Jackson, 2006). It is shown that publication bias can alter the estimation of the between-study heterogeneity estimate and hence, the variability of the pooled summary estimate. Jackson, 2006 has shown that it is impossible

to make generalizations concerning how we should revise estimates of between-study variance when presented with the possibility of publication bias and interpretation should proceed with cautiousness.

## Contour enhanced funnel plot

A later addition to the common funnel plot is a contour funnel plot (Peters et al., 2008). This is similar to the funnel plot but confidence interval lines, at usually 90, 95 and 99%, are added to the original funnel plot. The centre of the contour lines is the null, which could be zero or one depending on the pooled summary estimate. This is used as an aid to differentiating asymmetry due to publication bias from that due to other factors. If studies appear to be missing in areas of statistical non-significance, then this adds credence to the possibility that the asymmetry is due to publication bias. On the other hand, if the supposed missing studies are in areas of higher statistical significance, this would suggest that the cause of the asymmetry may be more likely to be due to factors other than publication bias as this would be inconsistent with the distribution of the study effects. Reasons for that could be increased heterogeneity, variable study quality, unknown confounding factors and others.

The contour enhanced funnel plot lines have to be distinguished from the pseudo 95% confidence intervals in a funnel plot. The later help us to see whether a study lies within the expected limits of the funnel plot and helps us to assess asymmetry and between-study heterogeneity. The former, helps us to visualize the distribution of the studies to assess whether the observed asymmetry is likely to be due to publication bias because of statistical significance or not. The 95% pseudo confidence intervals are determined by the studies in the meta- analysis and the pooled summary effect estimate and can be misleading when the observed pooled estimate is biased. The contour lines indicate levels of statistical

significance for the studies in the meta-analysis and are independent of the pooled summary effect estimate, and therefore not “pulled” by it because of bias. Both lines will coincide if the pooled effects estimate is the null.

## Galbraith radial plot

A Galbraith plot (also known as Galbraith's radial plot or just radial plot), is a graphical method of plotting effect estimates on their standard errors (Galbraith, 1998). The method exploits a familiar connection between standardized estimates and regression through the origin. It is a scatter plot of the SND against the inverse of the effect estimates standard error (horizontal axis). Larger studies (with smaller standard error and larger inverse standard error) will be observed to aggregate away from the origin, i.e. towards the right side of the plot. The regression model that is actually used is the following:

$$SND\ or\left(\frac{effect\ estimate}{SE}\right) = (pooled\ summary\ effect\ estimate) * \frac{1}{SE}$$

It is obvious from the above model that the slope of the line is equal to the pooled summary effect estimate and the origin is zero (from a zero SE). For 95% of studies, we expect this to be within 2 units of the true effect, if there is no heterogeneity. Trials outside the Galbraith limits will be trials where the 95% confidence interval does not contain the pooled summary effect estimate. Although, we can spot them from the forest plot, it is used to examine heterogeneity in a meta-analysis, as a supplement to the forest plot.

## Trim and Fill method

Another method, that was built upon the funnel plot technique is the trim and fill method (Duval and Tweedie, 2000). This is a nonparametric method for estimating the number of missing studies that might exist in a meta-analysis and the effect that these studies might have had on its outcome. This is usually the side where the positive or favourable results should be, which do not have negative counterparts. For most treatment effects, this is usually the right side. It uses rank-based data augmentation techniques. It calculates how many could be the missing studies and assigns a probable treatment effect estimate and variance to those studies. A revised pooled estimate "adjusting for publication bias" is then derived from this reduced dataset. Then, the "trimmed" studies are reinstated and the assumed missing studies are imputed on the opposite side of the funnel by "reflecting" the trimmed studies about the adjusted pooled summary effect line. One can then repeat the analysis by using the original studies and the new extrapolated missing studies and calculate a new summary effect. It is quite simple, easy to understand and it is suggested that it performs well. Duval and Tweedie, 2000 found that after adjusting for missing studies, the point estimate of the overall effect size is approximately correct and coverage of the effect size confidence intervals is substantially improved, in many cases recovering the nominal confidence levels entirely. In some cases the biases do not affect the conclusions but researchers should check routinely whether conclusions of systematic reviews are robust to possible non-random selection mechanisms (Sutton et al., 2000).

## Regression methods for funnel plot asymmetry

Regression methods are considered better in searching for heterogeneity and publication bias (Moreno et al., 2009). However, they are not graphical methods and less appealing to the non-statistician eye. Most of these are using the so-called standard normal deviate (SND) which is defined as the effect size divided by its standard error. The standard error is related to the size of the study and so the question these tests really ask is whether the treatment effect is related to the size of the study. The various methods differ in terms of the model (either a regular weighted regression with or a meta-analytic one is used), in terms of the SND where the predictor variable against which the observed outcomes are tested can be standard error, sampling variance, total sample size, or the inverse of the total sample size, and in terms of the outcome measure used (Viechtbauer, 2011). However, the idea behind the various tests is the same: if there is a relationship between the observed outcomes and the chosen predictor, then this usually implies asymmetry in the funnel plot and may be an indication of publication bias.

For parametric regression tests, an unweighted ordinary least squares (OLS) regression is estimated. When there is no evidence of funnel plot asymmetry, the intercept should not significantly differ from zero. In other words, the tests provide a measure of funnel plot asymmetry by examining whether the regression line (represented by the intercept) passes, in a statistically significant way, away from the origin (null) or not. Since the standard error depends on the sample size, the inverse of the standard error will be close to zero for small studies. Therefore, even though small studies may produce large effect sizes due to sampling error and publication bias, the SND will be small since the standard error will be large. The opposite will be true for large studies. Below we briefly explain the most common of the available methods:

The **rank correlation test** (Begg and Mazumdar, 1994) which is based on the rank correlation between standardised treatment estimates and variance estimates of the estimated treatment effects. The Kendall's tau rank correlation is used as correlation measure and the test statistic follows a standard normal distribution. The test statistic is a direct statistical analogue of the funnel plot. The test is fairly powerful for large meta-analyses with more than 75 studies, but has only moderate power for meta-analyses with less than 25 studies. However, in many of the configurations in which there is low power, there is also relatively little bias in the summary effect size estimate. Nonetheless, the test must be interpreted with caution in small meta-analyses.

The **Egger's test** (Egger et al., 1997) which is based on a weighted linear regression of the treatment effect on its standard error. Variations of this test use the variance instead of the standard error. The use of the variance as the predictor variable is supposed to provides less biased and more precise estimates than using the standard error. Another option of the test is by using the method of moments estimator for the additive between-study variance component. The test statistic follows a t distribution with degrees of freedom equal to the number of studies minus 2. Again, this is a test that must be interpreted with caution in small meta-analyses.

The **Harbord's test** (Harbord et al., 2006) is a test based on a weighted linear regression utilising the efficient score and its variance, Fisher's information. The test statistic follows a t distribution with degrees of freedom equal to the number of studies minus 2. A distinct advantage of this method is that it reduces the correlation between the log odds ratio and its corresponding standard error which causes asymmetry in funnels even when no small-study effects exist. It is considered to outperform Egger's test, almost in every case.

The **Peters' test** (Peters et al., 2006) is a test based on a weighted linear regression of the treatment effect on the inverse of the total sample size using

the variance of the average event rate as weights. It follows a t distribution with degrees of freedom equal to the number of studies minus 2. Peters et al.'s approach is a modification of Macaskill's test (Macaskill et al., 2001) and is supposed to work better than that. Using a function of the sample size as the predictor variable avoids the structural dependence between log odds ratio and its variance as seen in the other regression tests.

The **arcsine test** for funnel plot asymmetry (Rucker et al., 2008) is based on the arcsine transformation, which stabilizes the variance of binomial random variables. Rucker et al. developed this method mostly for odds ratios meta-analyses. It has slightly greater power, especially when the effect size is small and heterogeneity is present. Arcsine tests have additional advantages that they can include trials with zero events in both arms and that they can be very easily performed using the existing software for regression tests.

All methods perform less well with increasing unexplained heterogeneity and decreasing number of studies in a meta-analysis. Their power is low if the number of studies is less than 10 and there is not severe bias. This is an inherent problem of the tests since the failure of them to reject the null-hypothesis does not prove the null hypothesis true. The parametric regression method is generally more powerful than the non-parametric rank correlation method. According to Moreno et al., 2009 the variations of Egger’s test using the variance as a measure of precision and Peter’s test perform better than the others. However, Peter’s has the advantage of avoiding the structural correlation problem between outcome and standard error by using a function of sample size as the predictor variable and is the preferred method. In their analysis though, they did not test the arcsine test. Still there are some drawbacks for regression method: Use should be avoided when a substantial treatment effect is present (due to the universal effect on the SND) and there should be clear variation in study sizes. This is particularly a concern for the regression methods when all the studies are small, since a larger extrapolation to the intercept would be required. Finally,

remember that those tests do only assess funnel plot asymmetry and not for true publication bias or small study effects.

## Sensitivity analysis

One way of dealing with the problem is to do a sensitivity analysis. Sensitivity analysis is an analysis that is done after the original analysis. The purpose of a sensitivity analysis is to find out how robust are the data in the changing of various parameters or the data themselves. The most common method is to exclude the studies of the meta-analysis one by one and repeat the analysis (leave-one-out or influential meta-analysis). In the sequential approach the heterogeneity is estimated after the most influential study is removed. In combinatorial approach the heterogeneity is assessed after removing groups of studies (Patsopoulos et al., 2008). This is done in order to see the influence of each study in the summary effect estimate. If the statistical significance or the treatment effect do not change significantly after leaving a study out of the analysis, then that means that our data are robust enough. This possibility increases as the number of studies in the meta-analysis increases. If on the other hand do change, then this suggests that there exists a lack of homogeneity among the studies involved. However, this can also be seen grossly and quickly by looking at the weights that are assigned to each study. If those are assigned more or less equally between the studies, then usually none of them is so important as to affect the pooled summary effect on its own. We should note here that removal of large studies increases the average extent of within-study precision and thus reduces $I^2$, even though removal of such studies may actually increase the estimated amount of heterogeneity. This uncertainty is obvious when confidence intervals are estimated for $I^2$ (Viechtbauer, 2007).

Other methods include the addition of initially excluded studies because of incomplete data or data that they were imputated through various methods, or different assumptions about characteristics of the study. An analysis which is done by ignoring the missing data completely e.g. includes only the studies with available and good quality data is called “complete case analysis”. On the other hand, an analysis which includes incomplete cases where part of the missing variables (e.g. p-value or standard deviation) had to be imputated is called an “imputed case analysis” (Higgins and Altman, 2008). Higgins et al., 2008 have already reviewed and developed imputation methods for missing outcome data in meta-analysis of clinical trials with binary outcomes. They reviewed some common strategies, such as simple imputation of positive or negative outcomes, and developed a general approach involving informative missingness odds ratios' (IMORs). They also go on to describe several choices for weighting studies in the meta-analysis and give an example.

Finally, we could also apply meta-analysis to subsets of studies based on high-quality versus low-quality studies, randomized versus non-randomized studies, early studies versus late studies, etc. In any case, sensitivity analysis is essential and should address both estimates and weights.

## Cumulative meta-analysis

Cumulative meta-analysis is the way of performing the analysis by adding every study one by one to the analysis, starting from the earliest to the latest. This is done in order to show whether there is a consistency in the results and to find out the point at which no further study is necessary because the results robustly and continuously favour one treatment or the other. The most famous example is that of the use of streptomycin for thrombolysis, which could have been allowed almost 10 years before its official license.

## Meta-regression

Meta-regression is a logistic regression technique used to explore the relationship between study characteristics (e.g. concealment of allocation, baseline risk, timing of the intervention) and study results in a systematic review (The_Cochrane_Collaboration, 2005). This is a form of regression analysis that models an individual's odds of disease or some other outcome as a function of a risk factor or intervention. Meta-regression provides greater insight and a clearer interpretation of how study outcomes are affected by factors other than the primary treatment or study group. The covariate to be explored must be at least ordered (binary, ordinal, or interval) but not necessarily normally distributed.

## Individual Patient Data Meta-analysis

Meta-analysis of time-to-event data can also be done using individual patient data (IPD) and this is the most accurate method. A more thorough analysis, or rather re-analysis and investigation of heterogeneity is possible through that way (Smith et al., 2005). There are two ways that this can be performed. In one-stage analysis, all IPD data are combined in one Cox model with a variable identifying their origin (e.g. by author) and then stratified by that variable. In a two stage analysis, each trial is analysed separately for its HR and variance and then all are combined in the end. Recently the use of percentile ratios instead of hazard ratios has been proposed (Siannis et al., 2010; Barrett et al., 2012). IPD has many advantages and authors should be contacted for further details if possible.

### *Meta-analysis of time-to-event data*

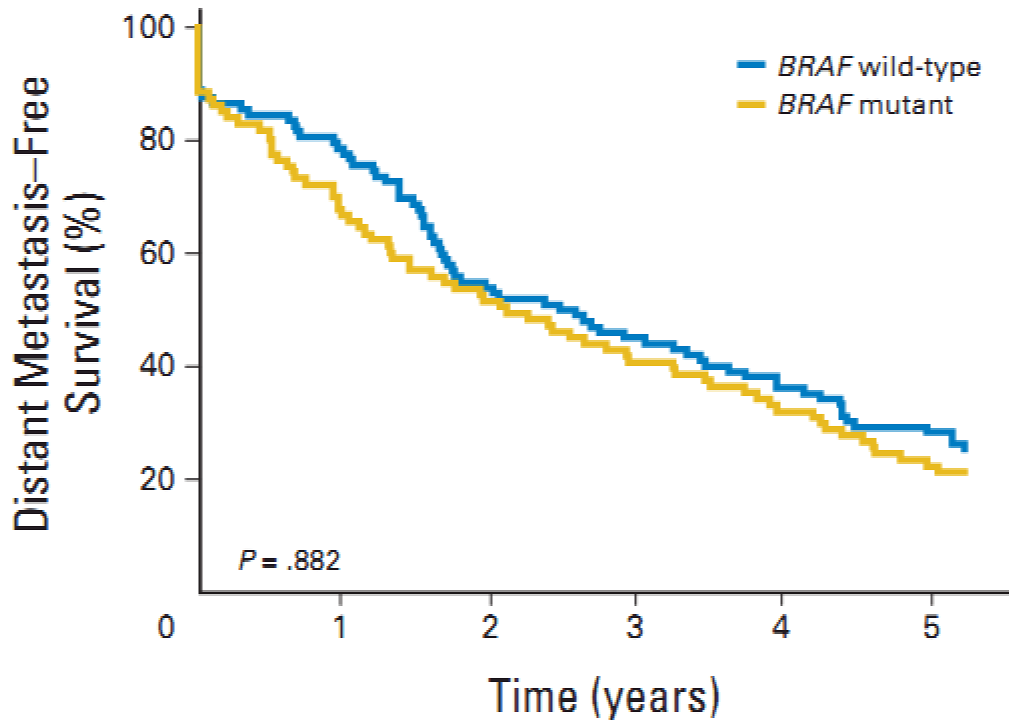
The data that we are called to collect in this meta-analysis are survival data or otherwise time-to-event data. These are data coming from measuring the length of time taken for an event to occur. The event needs to be clearly defined because there are different report outcomes for different situations. In our meta-analysis when the event is death from any cause, then the outcome reported is overall survival (OS). When the event is death from melanoma cancer or/and its complications then the outcome is melanoma specific survival (MSS). Generally, this is known as disease specific survival (DSS). Finally, when the event is a relapse or metastasis after full excision of the primary melanoma tumour, the outcome is disease free survival (DFS). Otherwise, this is known as progression free survival (PFS). Unfortunately, many researchers report overall survival (OS) when they mean disease specific survival (DSS) and the opposite. We did not find that in our study. However, there was one study where the outcome reported was referred as simply survival when in fact it was MSS (Janssen et al., 2008).

The time starting point needs also to be clearly defined. This can be the time of surgery, or the time of diagnosis, or the time of clinic appointment. In our study the time starting point is the time of excision of tissue specimen which led to the diagnosis of melanoma. Unfortunately, this can lead to even more confusion. In most of our included studies the time starting point is the time of diagnosis of primary tumour. Some studies though have as starting point the time of diagnosis of metastatic tumour (van Elsas et al., 1996; Ugurel et al., 2007; Caramuta et al., 2010). There is also one study where the starting point differs depending on the measured outcome (Long et al., 2011).

The reason for using survival analysis for time-to-event data is because the event may not be observed in all the patients. Some are lost to follow-up and for some others the research stops before the event has occurred. The only data available to us is the time that they were followed-up. In any other type of statistical

analysis these data would be lost. If we count those observations as having had the event then we will underestimate our average survival time, because more patients will have had the event than not, and consequently not "survived". If we ignore them, then we leave behind valuable information. But in survival analysis, these data can still be used and give information. The patients were the event has not occurred or observed are called censored. The time to event can be analysed as a binary response variable when the interest is in the event rather than the time to that event. That could be in cases where the event occurs early, is rare or the length of follow up is similar between patients. However, in cases where a lot of patients die or death comes after a long time then we get a more sensitive assessment by looking at the time to event and not the occurrence of event only.

The graphical display of the survival function is given by a Kaplan-Meier plot. The curve starts at 100% when all the patients are alive and it steps down each time an even occurs. In the following figure you can see an example of such a plot coming from one of our studies.




| No. at risk | | | | | | |
|---|---|---|---|---|---|---|
| *BRAF* wild-type | 102 | 79 | 55 | 46 | 37 | 29 |
| *BRAF* mutant | 93 | 62 | 48 | 38 | 30 | 22 |

**Figure 1:** Example of a Kaplan-Meier plot (from (Long et al., 2011)).

If all the patients reach the event before the end of the study then the curve reaches 0%. But if there are censored observations at the end of the study then the curve will end at a value above 0. In this case, the last censored observation and the maximum follow-up time correspond to the end of the curve. This is important to remember when we are trying to estimate minimum and maximum-follow up times from a Kaplan-Meier plot.

The curve is only an estimate of the real survival. We can calculate confidence intervals for the curve and that gives an estimate as to where the "real" survival curve would lie. The confidence interval gets wider towards the end of the curve because the numbers at risk (and hence the sample size) is getting smaller. Therefore, when interpreting a survival curve, it is always good practice to check

the numbers at risk and if they get too small, then we should interpret the results with caution.

One way of comparing the two groups is by the median survival time. The median survival time is the time beyond which 50% of the individuals in the population under study are expected to survive. Poor survival is reflected by a curve that drops relatively rapidly towards 0. However, this is not a good way to compare the two curves and we see below why.

The most common method of comparing the time to event for the two groups or otherwise the two survival groups is the logrank-test. It is non-parametric, takes censoring into account and compares the two groups for the whole time of follow-up (Tudur, 2010). That is why it should be preferred over the median survival time. The median survival time is actually comparing the two groups at the specific point in time beyond which 50% of the group are expected to survive. Therefore, it does not take into account the whole time period, or what happens to survival before or after that period. Moreover, for meta-analysis purposes, all the studies would have to report their data at the same time point. Michiels et al have assessed the accuracy of a meta-analysis when only the median survival times are known and concluded that these are not reasonable surrogate measures for meta-analyses of survival outcomes and that, wherever possible, HRs should be calculated (Michiels et al., 2005). The logrank test compares the total number of deaths observed with the number of deaths we would expect assuming that there is no group effect. It looks at whether the expected number of deaths is significantly different to the observed numbers in each group. If that is the case, then that is evidence that the group is associated with survival. It is obtained by constructing a 2x2 table at each distinct death time and comparing the death rates between the two groups, conditional on the number at risk in the groups. The table are then combined using the Cochran-Mantel-Haenszel test. Another name for the test is Cox-Mantel test. If $t_1, \ldots t_k$ represent the $k$ ordered,

distinct death times, then at the *j-th* time we have the following table (Williams and Yiannoutsos, 2009):

| Group | Die or Fail | Alive or Success | Total |
|---|---|---|---|
| 0 | $d_{0j}$ | $r_{0j} - d_{0j}$ | $r_{0j}$ |
| 1 | $d_{1j}$ | $r_{1j} - d_{1j}$ | $r_{1j}$ |
| Total | $d_j$ | $r_j - d_j$ | $r_j$ |

Where $d_{0j}$ and $d_{1j}$ are the number of deaths in group 0 and 1, respectively at the *j-th* death time, and $r_{0j}$ and $r_{1j}$ are the number at risk at that time in the same groups. The logrank test is given by the formula below:

$$x^2 logrank = \frac{\left[\sum_{j=1}^{k}\left(d_{0j} - \frac{r_{0j} * d_j}{r_j}\right)\right]^2}{\sum_{j=1}^{k}\left(\frac{r_{1j} * r_{0j} * d_j * (r_j - d_j)}{r_j^2 * (r_j - 1)}\right)} = \frac{\left[\sum_{j=1}^{k}(d_{0j} - e_j)\right]^2}{\sum_{j=1}^{k} v_j}$$

Where $v_j$ is the variance and $e_j$ the expected number of deaths. The logrank statistic depends on the ranks of event times only. The test is most powerful for proportional hazards. One empirical way to check for proportional hazards is to check if the survival curves cross. If they do, then this is evidence that the hazards are not proportional. If we want to compare more than two groups there are other variations of the logrank test or we can use a Wilcoxon-Gehan test.

Finally hazard and hazard ratios is something that we find often in survival analysis and is another way to compare two survival groups. The hazard is the chance that at any given moment, the event will occur, given that it hasn't already done so. The hazard ratio (HR) is a measure of the relative survival in two groups. It takes into account censoring and time to event data. It is the ratio of hazard for one group compared to another. The Cox proportional hazards model is used for its calculation.

In Cox proportional hazards regression model we assume that the hazard function is partly described by an underlying baseline hazard, and partly by the contribution of certain risk factors or covariates. The baseline hazard reflects the underlying hazard for subjects with all covariates $x_1,\ldots,x_p$ equal to 0. The model is given by the following equation:

$$h(t) = h_0(t) * \exp(b_1 x_1 + b_2 x_2 + \cdots + b_p x_p)$$

Let $x_1$=1 for the treatment group and $x_1$=0 for the control group and all other covariates are the same between the groups. The hazard ratio in this model is actually given by the exponential form of the coefficient of the characteristic or variable in question such as:

$$HR = \frac{h_0(t) * \exp(b_1 * 1)}{h_0(t) * \exp(b_1 * 0)} = \exp(b_1)$$

because the exponential form of 0 is 1. Therefore, the Cox proportional hazards model is a linear model for the natural logarithm of the hazard ratio, where we do not have to make any assumptions about the form of $h_0(t)$. For that reason the model is considered semi-parametric. When HR=1 then the hazard is the same in the two groups. When HR>1 then the group in question is at an increased hazard compared to the group in comparison. When 0<HR<1 then the group in question is at a decreased hazard compared to the group in comparison. We cannot have HR<0 as this the ratio of two hazards, and hazard is always a positive number. HR=0.5 means halving the risk and HR=2 means doubling the risk. The Cochrane handbook advises that the effect measure for time-to-event outcomes should be expressed as hazard ratio.

Suppose now that we want to calculate a pooled HR for all the studies in a meta-analysis. The most common method employed is the inverse variance method.

Suppose there are $k$ trials, and for each trial, $i$=1,2,…$k$, an estimate of the log hazard ratio and its variance are available. According to the generic inverse variance method, an estimate of the overall log hazard ratio across trials and its variance is given by:

$$\text{pooled } \ln(HR) = \frac{\sum_{i=1}^{k} \frac{\ln(HR_i)}{\text{var}[\ln(HR_i)]}}{\sum_{i=1}^{k} \frac{1}{\text{var}[\ln(HR_i)]}}$$

where

$$pooled\ var[\ln(HR)] = \left(\sum_{i=1}^{k} \frac{1}{\text{var}[\ln(HR_i)]}\right)^{-1}$$

Because a meta-analysis of HR is similar to a meta-analysis of OR or RR the method of Peto can also be used (Yusuf et al., 1985):

$$pooled\ lnHR = \frac{\sum_{i=1}^{k} logrank\ Observed\ events - logrank\ Expected\ events\ (O - E)}{\sum_{i=1}^{k} logrank\ Variance\ (V)}$$

Unfortunately, researchers very rarely publish all the necessary information (Hirooka et al., 2009). Therefore, methods needed to estimate the missing information from other available statistical data. In the last few years there have been two cardinal papers, by Palmar et. al. and Williamson et. al. where the authors explain a number of ways to extract summary statistics, namely the HRs, from published incomplete survival data (Parmar et al., 1998;Williamson et al., 2002). Tierney et. al. have gathered all the methods and they present them in a simple way in their article (Tierney et al., 2007). They have also published an excel file to be used for the calculations and it is the one we used for this meta-analysis and highly recommend. We briefly present the methods for generating

the Observed – Expected (*O-E*), Variance (*V*), *HR* and *ln(HR)* from reported summary statistics below, as well as a few comments for each method:

Following the notation of Parmar et al, 1998 we have:

For trial *i*:

$O_{ri}$ = observed number of events in the research group

$E_{ri}$ = logrank expected events in the research group

$O_{ci}$ = observed number of events in the control group

$E_{ci}$ = logrank expected events in the control group

$O_r$ - $E_r$ observed minus expected events in the research group

$O_i$ = total observed events ($O_{ri}$ + $O_{ci}$)

$V_{ri}$ = logrank variance

$ln(HR_i)$ = *log HR*

$var[ln(HR_i)$ = variance of the log hazard ratio

$UPPCI_i$ = Value for the upper end of the confidence interval

$LOWCI_i$ = Value for the lower end of the confidence interval

$\Phi^{-1}(1-\frac{a_i}{2})$ = z score for the upper end of the confidence interval

$R_{ri}$ = number randomised to the research group

$R_{ci}$ = number randomised to the control group

$p_i$= reported two-sided p-value associated with the logrank or Mantel-Haenszel test (or Cox model)

### *Estimating a pooled ln(HR) from a series of trials*

Estimating a pooled ln(HR) using the inverse variance method:

$$\ln(HR) = \frac{\sum_{i=1}^{k} \frac{\ln(HR_i)}{\mathrm{var}[\ln(HR_i)]}}{\sum_{i=1}^{k} \frac{1}{\mathrm{var}[\ln(HR_i)]}}$$

Estimating the *O-E, V, HR* and *lnHR* from reported summary statistics

$$\mathrm{var}(\ln(\mathrm{HR}_i)) = \frac{1}{V_{\mathrm{r}i}}$$

$$V_{\mathrm{r}i} = \frac{1}{\mathrm{var}(\ln(\mathrm{HR}_i))}$$

Directly estimating the *lnHR* and associated variance using the formal definition:

$$\ln(\mathrm{HR}_i) = \ln\left[\frac{O_{\mathrm{r}i} / E_{\mathrm{r}i}}{O_{\mathrm{c}i} / E_{\mathrm{c}i}}\right]$$

$$V_{ri} = \frac{1}{[(1/E_{\mathrm{r}i}) + (1/E_{\mathrm{c}i})]}$$

Direct estimation of the *lnHR* using an alternative definition:

$$\ln(\mathrm{HR}_i) = \left[\frac{O_{\mathrm{r}i} - E_{\mathrm{r}i}}{V_{\mathrm{r}i}}\right]$$

Indirect estimation of the variance of the *lnHR* from the confidence interval:

$$\mathrm{var}(\ln \mathrm{HR}_i) = \left[\frac{\mathrm{UPPCI}_i - \mathrm{LOWCI}_i}{2\Phi^{-1}(1 - \alpha_\mathrm{i} / 2)}\right]^2$$

Indirect estimation of the variance of the *lnHR* from the number of events:

$$V_{ri} = O_{ri}O_{ci}/O_i$$

$$V_{ri} = O_i/4$$

Indirect estimation of the variance of the *lnHR* from the number of events and the numbers randomised (analysed) on each arm:

$$V_{ri} = \frac{O_i R_{ri} R_{ci}}{(R_{ri} + R_{ci})^2}$$

Indirect estimation of the observed minus expected events from the observed events and the p-value:

$$(O_{ri} - E_{ri}) = \sqrt{\frac{O_{ri}O_{ci}}{O_i}} \times \Phi^{-1}(1 - \frac{p_i}{2})$$

$$(O_{ri} - E_{ri}) = 1/2 \times \sqrt{O_i} \times \Phi^{-1}(1 - \frac{p_i}{2})$$

Indirect estimation of the observed minus expected events from the observed events, the p-value and the numbers randomised (analysed) on each arm:

$$(O_{ri} - E_{ri}) = \frac{\sqrt{(O_i R_{ri} R_{ci}}}{(R_{ri} + R_{ci})} \times \Phi^{-1}(1 - \frac{p_i}{2})$$

***Generating the HR and V from published Kaplan-Meier curves and follow-up.***

Again, following the notation of Parmar et al., 1998, for trial *i* and T non-overlapping time points (t = 1, ...,T) :

$t$ = whole time interval $(t - 1, t)$

$t_s$ = start of the time interval $(t - 1, t)$

$t_e$ = end of the time interval $(t - 1, t)$

$R_{ri}(t)$ = effective number of patients at risk on the research arm during time interval $(t - 1, t)$

$R_{ri}(t - 1)$ = effective number of patients at risk on the research arm during time interval $(t - 2, t - 1)$

$D_{ri}(t)$ = effective number of events on the research arm during time interval $(t - 1, t)$

$D_{ci}(t)$ = effective number of events on the control arm during time interval $(t - 1, t)$

$D_{ri}(t - 1)$ = effective number of events on the research arm during time interval $(t - 2, t - 1)$

$C_{ri}(t)$ = effective number of patients censored on the research arm during time interval $(t - 1, t)$

$C_{ci}(t)$ = effective number of patients censored on the con-trol arm during time interval $(t - 1, t)$

$C_{ri}(t - 1)$ = effective number of patients censored on the research arm during time interval $(t - 2, t - 1)$

$S_{ri}(t_s)$ = event-free probability on the research arm at the start of time interval $(t - 1, t)$

$S_{ri}(t_e)$ = event-free probability on the research arm at the end of time interval $(t - 1, t)$

$F_{min}$ = minimum follow-up

$F_{max}$ = maximum follow-up

Estimation of the numbers event-free at the start of a time interval:

$$R_{ri}(t_s) = R_{ri}(t - 1) - D_{ri}(t - 1) - C_{ri}(t - 1)$$

Estimation of the numbers censored during a time interval:

$$\text{if } t_s \geq F_{\min} \text{ and } F_{\min} \leq t_e \leq F_{\max}$$
$$C_{ri}(t) = R_{ri}(t_s)\left\{\frac{1}{2}\frac{(t_e - t_s)}{(F_{\max} - t_s)}\right\} \text{ (assuming censoring at constant rate)}$$

Estimation of the numbers at risk during a time interval, adjusted for censoring:

$$R_{ri}(t) = R_{ri}(t_s) - C_{ri}(t)$$

Estimation of the number of events during a time interval:

$$D_{ri}(t) = \left[R_{ri}(t) \times \left(\frac{S_{ri}(t_s) - S_{ri}(t_e)}{S_{ri}(t_s)}\right)\right]$$

Note that the above equations are also are used for the control arm.

Estimation of the HR and V for a time interval from a Kaplan-Meier curve:

$$\ln(\mathrm{HR}_i(t)) = \ln\left(\frac{D_{ri}(t)/R_{ri}(t)}{D_{ci}(t)/R_{ci}(t)}\right)$$

$$\mathrm{var}[\ln(\mathrm{HR}_i(t))] = \frac{1}{D_{\tau i}(t)} - \frac{1}{R_{\tau i}(t)} + \frac{1}{D_{ci}(t)} - \frac{1}{R_{ci}(t)}$$

***Generating the HR and V from published Kaplan-Meier curves and the numbers at risk***

Now, following the notation of Williamson et. al., 2002 for time interval i:

$j$ = treatment group (where 1 = the control arm and 2= the research arm)

$t_i$-$1$ = time at the start of the current interval

$t_i$-$1$= time at the start of the prior interval

$n_{j,i}$= number at risk at end of interval $[t_i\text{-}1,t_i)$ in group $j$

$n_{j,i\text{-}1}$ = number at risk at start of interval $[t_i\text{-}1,t_i)$ in group $j$

$n^*_{j,i}$ = number at risk during interval $[t_i\text{-}1,t_i)$ in group $j$

$d^*_{j,i}$ = number of events during interval $[t_i\text{-}1,t_i)$ in group $j$

$c^*_{j,i}$ = number censored during interval $[t_i\text{-}1,t_i)$ in group $j$

$s^*_{j,i}$ = event-free probability at end of interval $[t_i\text{-}1,t_i)$ in group $j$

$s^*_{j,i\text{-}1}$ = event-free probability at start of interval $[t_i\text{-}1,t_i)$ in group $j$

$e^*_{j,i}$ = logrank expected events during interval $[t_i\text{-}1,t_i)$ in group $j$ = 2 (the research arm)

Estimation of the numbers at risk during a time interval from a Kaplan-Meier curve:

$$n^*_{j,i} = \frac{(n_{j,i-1} + n_{j,i})s^*_{j,i-1}}{(s^*_{j,i-1} + s^*_{j,i})}$$

Estimation of the number of events during a time interval from a Kaplan-Meier curve:

$$d_{j,i}^{*} = \frac{(n_{j,i-1} + n_{j,i})(s_{j,i-1}^{*} - s_{j,i}^{*})}{(s_{j,i-1}^{*} + s_{j,i}^{*})}$$

Estimation of the numbers censored during a time interval from a Kaplan-Meier curve:

$$c_{j,i}^{*} = \frac{2(n_{j,i-1}s_{j,i}^{*} - n_{j,i}s_{j,i-1}^{*})}{(s_{j,i-1}^{*} + s_{j,i}^{*})}$$

Estimation of the number of logrank expected events during a time interval from a Kaplan-Meier curve:

$$e_{2,i}^{*} = (d_{2,i}^{*} + d_{1,i}^{*}) * \frac{n_{2,i}^{*}}{n_{2,i}^{*} + n_{1,i}^{*}}$$

***Estimating or educated 'guesstimating' minimum and maximum follow-up***

Apart from the above we also need to estimate the minimum and maximum follow up. The following methods are recommended by Tierney et. al., 2007, when the minimum and maximum follow-up are not explicitly reported and provided that some indicators of extent of follow-up are given:

***For minimum follow-up, if the trial report presents:***

1. Censoring tick marks on Kaplan-Meier curve:

    Assume first tick mark indicates the point of minimum follow-up.

2. Median follow-up and accrual period:

Assume minimum follow-up = median follow-up minus half the accrual period.

3. Date of analysis and accrual period:

Assume minimum follow-up = date of analysis minus final date of accrual.

4. Date of submission and accrual period:

Assume estimated date of analysis = date of submission minus 6 months.

Assume minimum follow-up = estimated date of analysis minus final date of accrual.

***For maximum follow-up, if the trial report presents***

1. Censoring tick marks on Kaplan-Meier curve:

Assume last tick mark indicates the point of maximum follow-up

2. Median follow-up and accrual period:

Assume maximum follow-up = median follow-up plus half the accrual period.

3. Date of analysis and accrual period:

Assume maximum follow-up = date of analysis minus first date of accrual

4. Date of submission and accrual period:

Assume estimated date of analysis = date of submission minus 6 months.

Assume maximum follow-up = estimated date of analysis minus first date of accrual.

The direct methods are obviously the best and should be preferred. The formula for the alternate definition is as good as the formal one, but will start to divert once the number of events is less than 25. Results from a Cox regression model can also be used for a direct estimate of the *lnHR* and its variance. We should prefer the unadjusted *HRs* from a Cox model, as the studies will rarely have adjusted for the same variables and confounders. The p-value can come from a logrank test or a Cox regression model. However, in these cases, we will also need the number of events in both groups or the total observed number of events. If we have the number of events on each group instead of the total

observed events, and those are equal, then we can use the “balanced randomization” method of p-value that does not apply the numbers from randomization, to extract the summary statistics. If on the other hand, have unequal randomisation, or the randomisation was equal but the numbers were unequal due to various reasons, e.g. more patients were excluded in one group, then we can use the “unequal randomisation” p-value method, which applies the randomized patient numbers.

All 3 methods provide very close estimates to IPD, but the method of p-value and “balanced randomisation”, where the number of events for both groups are known is more precise for trials with low percentage of censoring (Tudur, 2010). The method of p-value with “unequal randomisation” is more precise for trials with unequal sample sizes.

For the extraction of data from survival curves, there are two methods. The first one is introduced by Parmar et el and estimates the number at risk (Parmar et al., 1998). The second one was suggested by Williamson, et al. and incorporates the numbers at risk (Williamson et al., 2002). Parmar, et al. (1998) assume constant censoring for the whole follow-up period and estimated the numbers at risk. Williamson et al incorporate the number at risk and do not assume constant censoring. We describe here the method by Parmar et al. For each trial we split the time-axis into T non-overlapping time intervals, chose so as to limit the number of events within any time interval. Then for each arm and time point, we read off the corresponding survival probability. The division is done in such a way, that the included number of events, in that interval, do not exceed the 20% of total number of events. However, the smaller the number of events, and the more the intervals, the better. Then on step 3, from reading the manuscript, we estimate the minimum and maximum follow-up time of the patients under investigation. This may either be given directly or has to be extracted or rather guessed. We can use the first and last censoring tick mark on the survival curves, or estimate it from the dates of accrual and date of submission, or

publication of the manuscript, as we have described earlier. The aim of the whole process is to estimate how many individuals where censored during that time period. On step 4 we calculate the NUMBER AT RISK at the START of the interval. We then calculate the NUMBER CENSORED DURING the interval and the NUMBER AT RISK DURING the interval. Finally, we calculate the NUMBER OF DEATHS DURING the interval. We then repeat the whole sequence of steps for the control group. After that we calculate the ln(HR) and its variance. This has to be done for all the intervals that we have decided to divide the survival curves. At the end we calculate the pooled ln(HR) and its variance for the trial by combining the estimates for all the intervals using the formula we presented above (see page 45).

When the estimated number of deaths within an interval on either arm is zero, we replace zero by a small number of deaths, usually $10^{-6}$, for that interval. According to Parmar et al, (1998) this provides the best estimate of the total number of deaths and overall variance in each arm. The intervals do not have to be all the same size in time, but they can be different according to the rate of events. So, we tend to have smaller intervals at the beginning of the survival curve, when we usually see more events and longer at the end of the curve. The survival curve approach, either by Parmar et al. (1998) or Williamson et al. (2002), eventually underestimates the variance. Therefore, those trials or studies, will tend to get more weight in the meta-analysis and may affect our summary effect. This is also supported by Parmar et al. (1998) on an empirical comparison of the various methods presented in his paper. Tudur et al. (2001) have all compared as many methods as possible across 24 trials from 2 systematic reviews (Tudur et al., 2001). In general, they found good agreement between the different methods and the data published. However, the survival curve approach is less reliable, especially when the event rate is low. They recommended looking at sensitivity analysis with different sets of follow-up range, if these have to be extracted from the curve or the context. D'Amico et al., (2000) also assessed the performance of the indirect methods and survival curves and their preliminary

results indicate that means and variances of the distribution of the *lnHRs* estimates derived through the indirect method were similar to those derived from individual data, regardless of the number of time intervals and the assumption of the maximum and minimum follow-up (D'Amico et al., 2000). This was our experience as well during this meta-analysis.

Obviously, when we attempt the extraction of summary statistics from published articles we are talking about lots of calculations and a mistake is quite possible if those are not automated. Therefore, we used the spreadsheet published by Tierney et al. (2007) and digitizer software. The output from the automated excel file can be seen below for one of the survival curves.

Trial ID: Ugurel_2007_OS_BRAF

10 Oct 2012 (21:01)
Output based on data available

Calculation method (see key)

| | Method | 1 | 2a | 2b | 2c | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Data? | No | No | No | No | Yes | No | No | Yes | No | No | Yes | Yes | No |
| **Summary data** | | | | | | | | | | | | | | |
| HR | 1.40 | na | na | na | na | 1.40 | na | na | 1.40 | na | na | 1.38 | 1.54 | na |
| Lower 95% CI | 0.80 | na | na | na | na | 0.71 | na | na | 0.87 | na | na | 0.85 | 1.00 | na |
| Upper 95% CI | 3.10 | na | na | na | na | 2.76 | na | na | 2.26 | na | na | 2.22 | 2.38 | na |
| ln(HR) | 0.34 | na | na | na | na | 0.34 | na | na | 0.34 | na | na | 0.32 | 0.43 | na |
| se(ln(HR)) | na | na | na | na | na | 0.35 | na | na | 0.24 | na | na | 0.24 | 0.22 | na |
| Variance | na | na | na | na | na | 8.37 | na | na | 16.85 | na | na | 16.85 | 20.32 | na |
| O-E | na | na | na | na | na | 2.82 | na | na | 5.67 | na | na | 5.38 | 8.79 | na |
| **Data Entered** | | | | | | | | | | | | | | |
| Lower CI (95%) | 0.80 | | | | | 0.80 | | | | | | | | |
| Upper CI (95%) | 3.10 | | | | | 3.10 | | | | | | | | |
| P value | 0.190 | | | | | | | | | na | na | 0.190 | | |
| Advantage to R or C? | C | | | | | | | | | na | na | C | | |
| Obs events Research | 39 | na | | | | | na | | | na | | | | |
| Obs events Control | 29 | na | | | | | na | | | na | | | | |
| Expt events Research | na | na | | | | | | | | | | | | |
| Expt events Control | na | na | | | | | | | | | | | | |
| Total events | 68 | | | | | | na | na | 68 | na | na | 68 | | |
| Pts analysed Research | 53 | | | | | | | | 53 | | | 53 | | |
| Pts analysed Control | 44 | | | | | | | | 44 | | | 44 | | |

**Key to methods**

**1** Report presents observed and expected events on research and control
**2a** Report presents HR and V
**2b** Report presents HR and O-E
**2c** Report presents O-E and V
**3** Report presents HR and CIs
**4** Report presents HR and events in each arm and randomisation ratio is 1:1
**5** Report presents HR and total events and randomisation ratio is 1:1
**6** Report presents HR, total events and the no.s analysed on each arm and randomisation ratio need not be 1:1
**7** Report presents p-value and events on each arm and randomisation ratio is 1:1
**8** Report presents p-value and total events and randomisation ratio is 1:1
**9** Report presents p-value, total events & no.s analysed on each arm and randomisation ratio need not be 1:1
**10** Data from curve read where wished and assuming constant censoring
**11** Data from curve with numbers at risk given (1)

**Key to table cells**

Data — Data fields inputted into (1)_Summary_Data sheet
Data — Data fields calculated from inputted data

| | Min | Max | Range |
|---|---|---|---|
| HR | 1.38 | 1.54 | 0.17 |
| V | 8.37 | 20.32 | 11.95 |
| O-E | 2.82 | 8.79 | 5.97 |

Print page

**Figure 2: Output of calculated *HRs* and other values through various available methods and after the extraction of data.**

There are several digitizer programs in the market but we used the one by DigitizeIt ( http://www.digitizeit.de/) with no problems at all {Bormann, #363}. Very few authors have mentioned using digitizer software to extract data

(Jansen, 2011; Guyot et al., 2012). Each survival curve is extracted separately. We then combine the exported by the digitizer software data into a different spread sheet and finally into the spread sheet made by Tierney et al. 32 survival curves were extracted that way from 16 different K-M plots. In the figures below, you can see the extraction of one survival curve using the digitizer software and the reconstructed survival curve. The crosses represent the points where time divides or otherwise the limits of the time intervals. We first need to define the limits and the metrics of x-axis and y-axis. This is usually in months for x-axis and from 0 to 100 for y-axis. The program gives us the length in points and therefore, for the x-axis, we have to calculate how many points correspond to every month, so that we can divide the time in equal intervals and to know where these points refer to. We have to use the same intervals, or otherwise place the crosses on the curve, at the same co-ordinates for both survival curves. The program gives us the corresponding values on x and y-axis in a table on the right. This table can be exported in a csv file and imported in excel where we can do our final calculations (see Figure 3). Note that survival cannot have “ups” and “downs”. Therefore, before importing to excel we have to make sure that all the values of survival are in fact decreasing with time and that they remain the same for parts of the curve that are straight horizontal lines parallel to x-axis. This is important because the program is very sensitive and it is very easy to find yourself 1 or 2 points above to where you should be.

For the meta-analysis, we used the software R (Team, 2008), available at: http://www.r-project.org, which is a freeware and open-source program and packages “metaphor” (Viechtbauer, 2011), “meta” (Schwarzer, 2012) and “survival”. Version 2.15.2 was used.

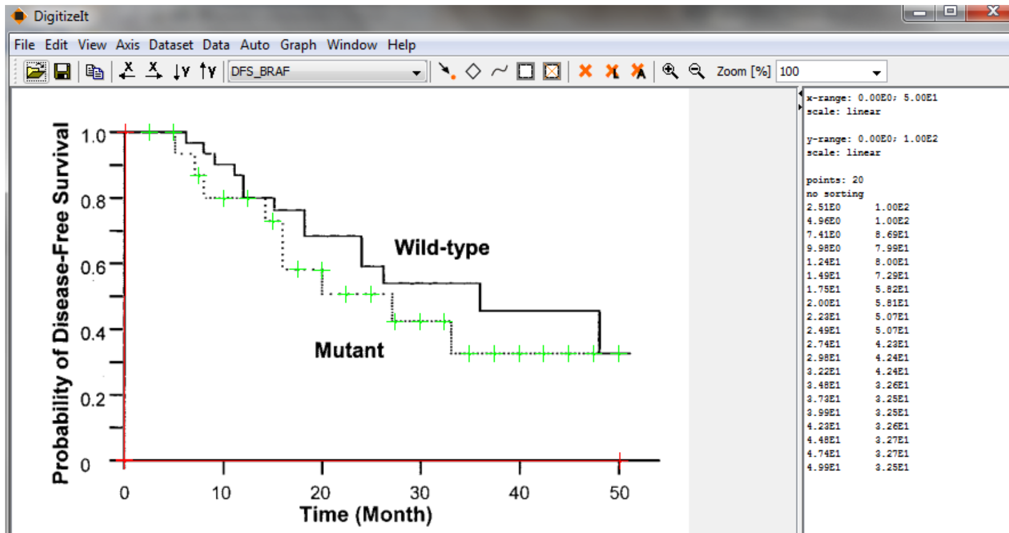



**Figure 3: Extraction of wild type BRAF DFS survival for melanoma patients from Shinozaki et al., 2004**

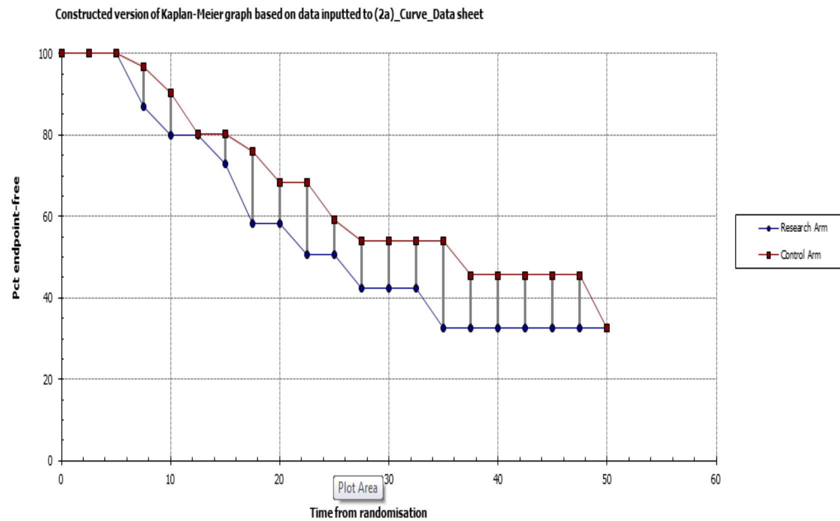



**Figure 4: The reconstructed survival curve from above, in Excel, having used the digitizer software before, for the extraction of data.**

| | A | B | C | D | E |
|---|---|---|---|---|---|
| 1 | DigitizeIt Value | DigitizeIt Month | Month | DFS BRAF | DFS WT |
| 2 | 98, 326 | 0 | 0 | 100 | 100 |
| 3 | 120 | 2.507151933 | 2.5 | 100 | 100 |
| 4 | 141 | 4.956717199 | 5 | 100 | 100 |
| 5 | 163 | 7.413857018 | 7.5 | 86.87099 | 96.87983599 |
| 6 | 185 | 9.982708951 | 10 | 79.91289 | 90.26970926 |
| 7 | 206 | 12.40340564 | 12.5 | 79.91289 | 80.09116551 |
| 8 | 228 | 14.94353948 | 15 | 72.87191 | 80.09116551 |
| 9 | 250 | 17.48803747 | 17.5 | 58.1679 | 76.09651686 |
| 10 | 271 | 19.96647132 | 20 | 58.1679 | 68.35487232 |
| 11 | 292 | 22.33384516 | 22.5 | 50.69157 | 68.35487232 |
| 12 | 314 | 24.86986567 | 25 | 50.69157 | 59.05116435 |
| 13 | 335 | 27.35306496 | 27.5 | 42.34521 | 54.00741504 |
| 14 | 357 | 29.83139848 | 30 | 42.34521 | 53.92484739 |
| 15 | 378 | 32.22332691 | 32.5 | 42.34521 | 53.92484739 |
| 16 | 400 | 34.82265263 | 35 | 32.61061 | 53.92484739 |
| 17 | 422 | 37.30108647 | 37.5 | 32.61061 | 45.58280667 |
| 18 | 444 | 39.8659254 | 40 | 32.61061 | 45.58280667 |
| 19 | 465 | 42.28662209 | 42.5 | 32.61061 | 45.58280667 |
| 20 | 487 | 44.82259243 | 45 | 32.61061 | 45.58280667 |
| 21 | 509 | 47.38743136 | 47.5 | 32.61061 | 45.58280667 |
| 22 | 531 | 49.92355219 | 50 | 32.61061 | 32.61060728 |
| 23 | | | | | |

**Figure 5: The data from the extraction of the two curves from figure (3).**

## *METHODOLOGY*

### Study eligibility and identification

We performed systematic computerized searches of the MEDLINE (through PubMed), and SCOPUS databases (from inception to 31$^{st}$ October 2012); and the Cochrane library to identify all published articles reporting on *KRAS*, *NRAS*, *HRAS* and *BRAF* somatic mutation analysis in all cancers. Combinations of the following keywords and their synonyms were used in the search engine: *"KRAS",*

*"NRAS", "HRAS", "BRAF", "cancer", and "RAS".* No language restriction was imposed. We also hand-searched journals known to publish data relevant to our topic, the reference lists of all retrieved articles and those of relevant review articles were also cross-referenced. Experts in the field were contacted to broaden the yield of our search.

The search was conducted in two rounds. In the first round: eligible studies were those that reported on search terms at the level of a citation. 23181 articles were assessed for eligibility. In the second round: full articles were extracted for these and eligible studies from this pool were those that performed somatic mutational analysis of the genes of interest (using any technique) with cancer, and this was sub-grouped by melanoma. 177 articles were extracted from this second round and were reviewed fully. This series was then divided into articles that presented or allowed the calculation of hazard ratios (HRs) with corresponding 95% confidence intervals (CIs) comparing overall survival (OS) stratified by *BRAF* or *NRAS*, for patients' naïve to treatment. Whenever multiple reports pertained to overlapping groups of patients, we retained only the report with the longest follow-up (largest number of events) to avoid duplication of information. 6 articles were excluded because of duplicate data. All study designs were considered potentially eligible as long as they provided adequate survival information, whether they were single or multiple arm trials, randomized or non-randomized, prospective or retrospective. 116 studies were excluded because they had no survival data. Studies examining treatment related outcomes were screened to indicate potential placebo controlled arms, or populations receiving any of the following: neo-adjuvant chemotherapy, post-surgical adjuvant therapy, radiotherapy (pre- or post-surgically), and those in which less than 20% of the entire population received any additional treatment strategy other than surgical resection (including: concomitant chemo-radiotherapy, chemotherapy, targeted agents, or biological). 4 articles were excluded because of reporting only patients who received treatment other than surgical. 16 case series, defined as studies reporting on 15 or fewer patients, were excluded. 3 reviews were also excluded.

Of the remaining articles, 1 found no mutation, 1 found only one mutation and 1 had only paediatric patients. 6 had not enough data or published survival curves to extract the necessary hazard ratios or measured survival comparing characteristics other than the gene mutations. Finally, 11 articles satisfied our criteria and were used in various combinations depending on their available data and reported outcomes (Figure 6).

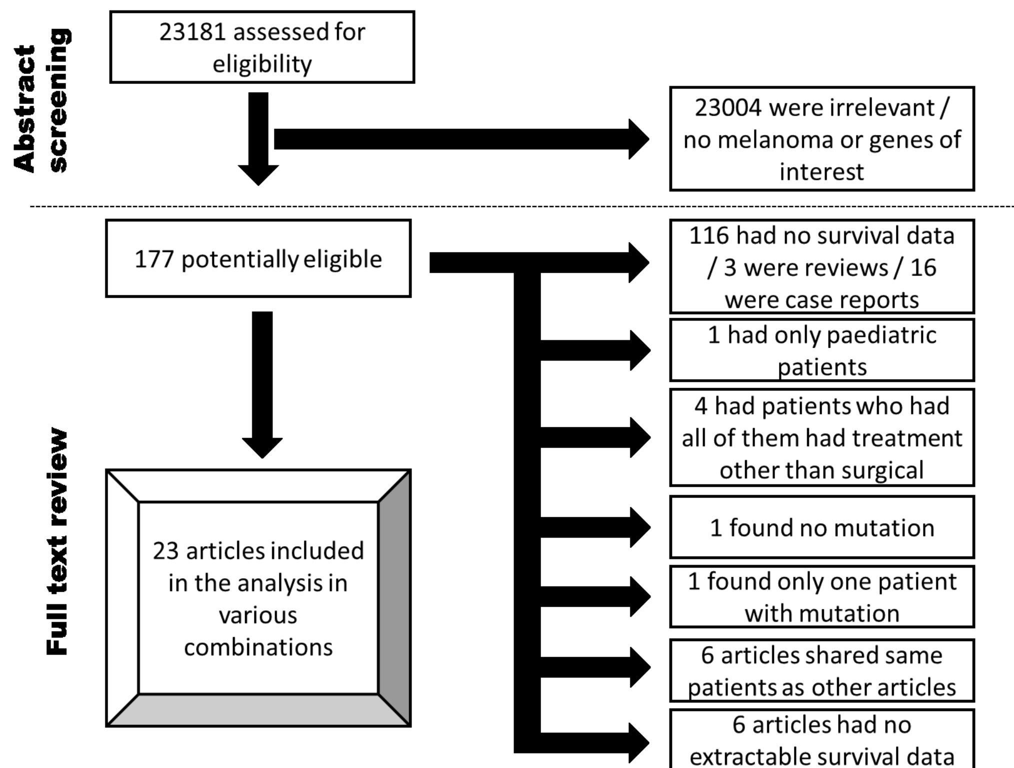



**Figure 6: Search strategy and study eligibility flow chart.**

Our literature search was limited to published studies, however, when datasets were incomplete for required data corresponding authors were contacted on a planned schedule of timed follow-up and data set closure. This author contact addressed the two series of articles screened above; a) those in which incomplete data was available in the manuscript; and b) otherwise eligible manuscripts that did not report any relative treatment or survival data. 20 authors were contacted out of which only 2 provided additional information.

Out of all the studies that were excluded, we feel obliged to present the reasons for excluding 8 studies with possible survival data. Their characteristics and reason for exclusion can be found summarized in table (1).

**Table 1: Excluded studies with possible survival data and reason.**

| Main Author | Year | Locality of samples | Reason for exclusion |
|---|---|---|---|
| Chang | 2004 | North America | No survival data. |
| Houben | 2004 | Europe | BRAF and NRAS are combined in one arm. |
| van Dijk | 2005 | Europe | Unable to distinguish between mutated and wild type patients from data reported for other outcome. |
| Shinozaki | 2007 | North America | He measures only serum BRAF, not tissue BRAF. |
| Johansson | 2009 | Europe | Two isolated survival groups (<14months and >60) No continuous survival data but only binary data between the two distinct groups. |
| Davies | 2009 | North America | Survival data (KM curves) is not on BRAF or NRAS but P-AKT. |
| Sviatoha | 2010 | Europe | Inappropriate statistical analysis of survival data and no available results. |
| Ellerhosrst | 2011 | North America | Unfortunately, in his only one survival curve, combines NRAS and BRAF into one category and compares both together with wild type patients. Also the numbers at risk are far less than the other numbers in the article and so numbers and events cannot be extracted that way. |

## Study characteristics and assessment of bias

As we mentioned on our first theoretical part of this thesis, assessment of the characteristics of the studies is essential before any analysis. The reason for that is to assess the heterogeneity, to assess for bias and to decide on a fixed or random-effects model before any statistical test takes place. In the following tables we present the characteristics of all the included studies, as well as those that were used only for sensitivity analysis. It is easily seen by the following tables how different are our included studies between them. They differ in their origin, specimen types, melanoma types, stages included, BRAF and NRAS exons and codons analysed, methods of gene extraction and analysis, starting point of measurement of survival. Their heterogeneity is explored more in the statistical analysis where both fixed and random-effects analyses results are presented. It is obvious that the random effects should be the more appropriate for our collection of studies.

We also present a summary of the assessment of bias for the studies and the bias assessment for all the studies. We explored these features further in our analysis and there is an argument as to whether we should include only the high quality studies. However, that would almost half our included number of studies in most cases. On the other hand, as explained in the analysis, it is certain differences between the studies, like the starting point of the survival measurement and the melanoma stages included, that matter more than anything else.

**Table 2: Included study characteristics - part 1.**

| Main Author | Year | Continent | Countries | Study Design | Time where survival starts from | Type of survival measured | Mutation examined | IPD available |
|---|---|---|---|---|---|---|---|---|
| Broekaert | 2010 | North America and Europe | USA and Europe | Retrospective | The date of biopsy of primary tumour. | DFS, OS | BRAF | No |
| Devitt | 2011 | Oceania | Australia | Mixed | The date of biopsy of primary tumour. | DFS, OS, MSS | BRAF, NRAS | No |
| Shinozaki | 2004 | North America | USA | Mixed | The date of biopsy of primary tumour. | DFS | BRAF | No |
| Janssen | 2008 | Europe | UK | Retrospective | The date of biopsy of primary tumour. | MSS | BRAF | No |
| Omholt (only for sensitivity analysis as the data are included in the Edlundh-Rose) | 2003 | Europe | Sweden | Retrospective | The date of biopsy of primary tumour. | OS | BRAF, NRAS | No |
| Edlundh-Rose (Includes Omholt 2003 and Caramuta 2010) | 2006 | Europe | Sweden | Retrospective | The date of biopsy of primary tumour. | OS | BRAF, NRAS | No |
| Deichmann | 2004 | Europe | Germany | Retrospective | The date of biopsy of primary tumour. | DFS | BRAF | Yes (limited) |
| Long | 2011 | Oceania | Australia | Prospective | The date of biopsy of primary tumour for DFS and metastatic for OS. | DFS, OS | BRAF | No |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Ugurel | 2007 | Europe | Germany | Prospective | The date of biopsy of metastatic tumour. | OS | BRAF, NRAS | Yes |
| Caramuta (includes Danioti, is included in Edlund-Rose) | 2010 | Europe | Sweden | Retrospective | The date of biopsy of metastatic tumour. | OS, MSS | BRAF, NRAS | Yes (limited) |
| Elsas | 1996 | Europe, Africa and Oceania | Australia and Netherlands (3 continents) | Retrospective | The date of biopsy of metastatic tumour. | OS | NRAS | No |
| Omholt | 2002 | Europe | Sweden | Retrospective | The date of biopsy of primary tumour. | OS | NRAS | No |
| Demunter (codons 12 and 61 with x2 test) | 2001 | Europe | Belgium | Retrospective | The date of biopsy of primary tumour. | DFS | NRAS | Yes (limited) |
| Pouryazdanparast | 2012 | North America | USA | Retrospective | The date of biopsy of primary tumour. | DFS, MSS | BRAF, NRAS | Yes (limited) |
| Jacob | 2011 | North America | USA | Retrospective | The date of biopsy of metastatic tumour. | OS | BRAF, NRAS | No |
| Brown | 2012 | Europe | UK | Retrospective | The date of biopsy of primary tumour. | DFS, OS, MSS | BRAF | No |
| Lu (HR from IPD) | 2012 | Asia | China | Retrospective | The date of biopsy of primary tumour. | OS | BRAF, NRAS | Yes |
| Akslen | 2005 | Europe | Norway | Retrospective | The date of biopsy of primary tumour. | OS, MSS | BRAF, NRAS | Yes |
| Menzies (Includes Long) | 2012 | Oceania | Australia | Prospective | The date of biopsy of primary tumour for DFS and metastatic for MSS. | DFS, MSS | BRAF | No |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Turri | 2012 | Europe | Italy | Retrospective | The date of biopsy of primary tumour. | DFS, MSS | BRAF ?, NRAS | Yes |
| Takata | 2007 | Asia | Japan | Retrospective | The date of biopsy of primary tumour. | DFS, OS | BRAF, NRAS | Yes |
| Casula | 2004 | Europe | Italy | Retrospective | The date of biopsy of primary tumour. | DFS, OS | BRAF | Yes |
| Kumar | 2003 | Europe | Sweden | Retrospective | The date of biopsy of primary tumour. | DFS | BRAF, NRAS | Yes |

**Table 3: Included study characteristics - part 2.**

| Main Author | Inclusion and Exclusion criteria | Only metastatic patients | Specimen Type | Analysis type | Fixation Type |
|---|---|---|---|---|---|
| Broekaert | Minimum 3yr follow up in order to be included. No exclusion criteria. However, the minimum follow-up is less from KM curves, so this is wrongly reported. | 0 | Primary Cutaneous Melanoma. | Tissue biopsy | Formalin fixed, paraffin embeded |
| Devitt | 2 out 251 were excluded because it was not possible to get the mutation status. All clinical stages are included. | 0 | Primary Cutaneous Melanoma. | Tissue biopsy | Paraffin embedded |
| Shinozaki | No exclusion criteria | 0 | Primary Cutaneous Melanoma. | Tissue biopsy | Paraffin embedded |

| | | | | | |
|---|---|---|---|---|---|
| Janssen | Only choroidal (occular) melanoma cases included | 0 | Primary occular (choroidal) melanoma | Tissue biopsy | Formalin- or gluteraldehyde-fixed, paraffin-embedded |
| Omholt (only for sensitivity analysis as the data are included in the Edlundh-Rose) | Only patients whose disease had progressed to metastatic were included in this study. | 1 | Primary (apart from 2 which were metastatic) | Tissue biopsy | Paraffin embedded |
| Edlundh-Rose (Includes Omholt 2003 and Caramuta 2010) | Only patients whose disease had progressed to metastatic were included in this study. | 1 | Primary (39) and metastatic (255) | Tissue biopsy | Fresh frozen and paraffin embedded |
| Deichmann | Only pairs of cancerous and non-cancerous tissue from the same patient included. | 0 | Primary cutaneous melanoma | Tissue biopsy | Paraffin embedded |
| Long | Ocular melanoma is excluded. Only metastatic stage IIIC or IV are included. All included patients had metastatic disease. The data on primary were found retrospectively. | 1 | Distant metastatic tissue (111), locoregional (61) and primary (23) | Tissue biopsy | Paraffin embedded |
| Ugurel | Primary ocular melanomas were excluded. Only stages III and IV were included with confirmed metastatic disease. | 1 | Solid metastatic lesion or malignant effusion. | Tissue biopsy | Fresh frozen |
| Caramuta (includes Danioti, is included in Edlund-Rose) | No exclusion criteria | 0 | Lymph node metastasis | Tissue biopsy | Paraffin embedded |
| Elsas | No exclusion criteria | 0 | Primary and metastatic melanoma | Tissue and cell lines | Paraffin embedded |
| Omholt | Only patients whose disease had progressed to metastatic were included in this study. | 1 | Primary (apart from 2 which were metastatic) | Tissue biopsy | Paraffin embedded |
| Demunter (codons 12 and 61 with x2 test) | Patients who had at least 6 months of follow up and were stages I and II. | 0 | Primary | Tissue biopsy | Fresh frozen and paraffin embedded. |
| Pouryazdanparast | Only cases who had gains of 8q24. They were also all primary melanomas with no evidence of metastasis at the time of biopsy. | 0 | Primary | Tissue biopsy | Paraffin embedded |

| | | | | | |
|---|---|---|---|---|---|
| Jacob | Only nonuveal, metastatic melanoma who underwent molecular testing within 6 months of their stage IV. | 1 | Not mentioned but probably metastatic | Not mentioned | Not mentioned |
| Brown | Patients were excluded if tissue blocks were unavailable or there was insufficient tissue for scoring as judged by the pathologist | 0 | Primary and metastatic melanoma | Tissue biopsy | Paraffin embedded |
| Lu (HR from IPD) | No exclusion criteria | 0 | Primary | Tissue biopsy | Paraffin embedded |
| Akslen | No exclusion criteria | 0 | Primary | Tissue biopsy | Paraffin embedded |
| Menzies (Includes Long) | Ocular melanoma is excluded. Only metastatic stage IIIIC or IV are included. All included patients had metastatic disease. The data on primary were found retrospectively. | 1 | Distant metastatic tissue, locoregional and primary | Tissue biopsy | Paraffin embedded |
| Turri | No exclusion criteria. Only sinonasal tumours are included. Only one BRAF mutation | 0 | Primary | Tissue biopsy | Paraffin embedded |
| Takata | No exclusion criteria | 0 | Primary | Tissue biopsy | Paraffin embedded |
| Casula | No exclusion criteria | 0 | Primary and metastatic melanoma | Tissue biopsy | Unknown |
| Kumar | Only metastatic melanoma patients | 1 | Unknown but probably primary and metastatic melanoma | Tissue biopsy | Fresh frozen |

**Table 4: Included study characteristics - part 3.**

| Main Author | DNA or RNA | Treatment Status | Radiotherapy | Mutation BRAF | Exons BRAF | Technique BRAF |
|---|---|---|---|---|---|---|
| Broekaert | DNA | No mention of treatment status. | Not mentioned. | No mention of mutation type. | 15 | Sequence |
| Devitt | DNA | No mention of treatment status. | Not mentioned. | V600E | 15 | Duplex allele specific PCR |
| Shinozaki | DNA | No mention of treatment status. | Not mentioned. | V600E, V600G | 11+15 | Taq high-fidelity polymerase PCR |
| Janssen | DNA | No mention of treatment status | Not mentioned. | T1799A | 15 | Nested PCR |
| Omholt (only for sensitivity analysis as the data are included in the Edlundh-Rose) | DNA | No mention of treatment status. | Not mentioned. | V600G, V600A, V600L, G468S, L600G, 6-bp ins | 11+15 | Single strand conformation polymorphism (SSCP-Seq) |
| Edlundh-Rose (Includes Omholt 2003 and Caramuta 2010) | DNA | No mention of treatment status | Not mentioned. | 600 (various) | 11+15 | Pyrosequencing |
| Deichmann | DNA | No mention of treatment status | Not mentioned. | V599E | 15 | Single strand conformation polymorphism (SSCP-Seq) |
| Long | DNA | Some patients had systemic treatment including BRAF inhibitor after the diagnosis of metastasis. This does not affect the research as they were separated on doing the calculations. | Not mentioned. | V600E, V600K and others | 15 | HRM Seq (High Resolution Melting Analysis using primers) |

| | | | | | | |
|---|---|---|---|---|---|---|
| Ugurel | DNA | Both with and without systemic treatment included with no discrimination. However there is no mention of BRAF inhibitor. | Not mentioned. | V600E, V600K, G469V, G469R, D594N | 11+15 | Fluorescent and radioactive single strand conformation polymorphism (SSCP-Seq) |
| Caramuta (includes Danioti, is included in Edlund-Rose) | DNA | No mention of treatment status | Not mentioned. | V600K, V600E | 15 | Single strand conformation polymorphism (SSCP-Seq) |
| Elsas | DNA | No report of treatment status | Not mentioned. | N/A | N/A | N/A |
| Omholt | DNA | No mention of treatment status. | Not mentioned. | N/A | N/A | N/A |
| Demunter (codons 12 and 61 with x2 test) | DNA | No mention of treatment status. | Not mentioned. | N/A | N/A | N/A |
| Pouryazdanparast | DNA | No mention of treatment status. | Not mentioned. | V600E | 15 | Sequence |
| Jacob | DNA | Some patients had immunotherapy and inhibitors and are analysed seperately. | Not mentioned. | V600E, V600K | 15 | Pyrosequencing |
| Brown | DNA | Not mentioned but unlikely to include inhibitors as the patients included were between 1993 and 1997. | Not mentioned. | V600E | 15 | Nested PCR |
| Lu (HR from IPD) | DNA | Not mentioned but inhibitors were not given. | Not mentioned. | Various | 11+15 | PCR |
| Akslen | DNA | Not mentioned but inhibitors were not given. | Not mentioned. | Various | 15 | Single strand conformation polymorphism (SSCP-Seq) |
| Menzies (Includes Long) | DNA | Some patients had systemic treatment including BRAF inhibitor after the diagnosis of metastasis. This does not affect the research as they were separated on doing the calculations. | Not mentioned. | V600E, V600K and others | 15 | HRM Seq (High Resolution Melting Analysis using primers) |

| | | | | | | |
|---|---|---|---|---|---|---|
| Turri | DNA | Treatment is mentioned. Some had chemotherapy and some radiotherapy. No-one had inhibitors. | Some had radiotherapy | D594G | 15 | Sequence |
| Takata | DNA | Not mentioned but inhibitors most likely were not given. | Not mentioned. | V600 mutations | 15 | Direct sequence |
| Casula | DNA | Not mentioned but inhibitors most likely were not given. | Not mentioned. | Various | 3+15 | Denaturing high performance liquid chromatography (DHPLC) and sequence |
| Kumar | DNA | Chemoimmunotherapy. No inhibitors. | Not mentioned. | Various | 1+11+15 | Single strand conformation polymorphism (SSCP-Seq) |

**Table 5: Included study characteristics - part 4.**

| Main Author | Codons NRAS | Mutation NRAS | Exons N-RAS | Technique NRAS |
|---|---|---|---|---|
| Broekaert | Not mentioned | No mention of mutation type. | Not mentioned | Sequence |
| Devitt | 61 | Q61L | 3 | High resolution melting PCR |

| | | | | |
|---|---|---|---|---|
| Shinozaki | N/A | N/A | N/A | N/A |
| Janssen | N/A | N/A | N/A | N/A |
| Omholt (only for sensitivity analysis as the data are included in the Edlundh-Rose) | 61 | G61L, G61H, G61A, G61Le | Not mentioned (2 from 2002) | Single strand conformation polymorphism (SSCP-Seq) |
| Edlundh-Rose (Includes Omholt 2003 and Caramuta 2010) | 61 | 61 (various) | 2 | Pyrosequencing |
| Deichmann | N/A | N/A | N/A | N/A |
| Long | N/A | N/A | N/A | N/A |
| Ugurel | 12(1), 13(1), 61(2), 68(2) | G12D, G13D, G13R, Q61R, G61L, Q61H, Q61K, R68T | 1+2 | Single strand conformation polymorphism (SSCP-Seq) |
| Caramuta (includes Danioti, is included in Edlund-Rose) | 61 | Q61R, Q61L, Q61K | 2 | Single strand conformation polymorphism (SSCP-Seq) |
| Elsas | 12 (1), 13(1), 61(2) | Various | 1+2 | SSCP gel electrophoresis |
| Omholt | 61 | G61L, G61H, G61A, G61Le | 2 | Single strand conformation polymorphism (SSCP-Seq) |
| Demunter (codons 12 and 61 with x2 test) | 12(1), 13(1), 61(2) | Various | 1+2 | SSCP gel electrophoresis (DGGE) |

| | | | | |
|---|---|---|---|---|
| Pouryazdanparast | 12, 13, 61 | Q61R, Q61L | 2+3 | Sequence |
| Jacob | 12, 13, 60, 61 | Q61R/K/L | 1+2 | Pyrosequencing |
| Brown | N/A | N/A | N/A | N/A |
| Lu (HR from IPD) | 12, 13, 61 | Various | 1+2 | PCR |
| Akslen | 12, 13, 62 | Various | 1+3 | Single strand conformation polymorphism (SSCP-Seq) |
| Menzies (Includes Long) | N/A | N/A | N/A | N/A |
| Turri | 12, 13, 61 | Various | 1+2 | Sequence |
| Takata | 61 | Various | 3 | Direct sequence |
| Casula | N/A | N/A | N/A | N/A |
| Kumar | 61 | Various | 1+2 | Single strand conformation polymorphism (SSCP-Seq) |

**Table 6: Included study characteristics - part 5.**

| Main Author | Samples with Mutation - BRAF | Total Sample Number | Samples with Mutation - NRAS | Total Sample Number | Follow-up time min | Follow-up time max |
|---|---|---|---|---|---|---|
| Broekaert | 159 | 288 | 51 | 288 | 0.759415092 | 142.3796386 |
| Devitt | 112 | 249 | 36 | 249 | 0 | 63 |
| Shinozaki | 18 | 59 | Not tested | Not tested | 1 | 87 |
| Janssen | 11 | 30 | Not tested | Not tested | 4 | 344 |
| Omholt (only for sensitivity analysis as the data are included in the Edlundh-Rose) | 42 | 71 | 21 | 71 | Unknown | Unknown |
| Edlundh-Rose (Includes Omholt 2003 and Caramuta 2010) | 120 | 219 | 61 | 219 | 2.3 | 300 |
| Deichmann | 19 | 50 | Not tested | Not tested | 2 | 77 |

| | | | | | | |
|---|---|---|---|---|---|---|
| Long | 95 | 197 | Not tested | Not tested | 0.2 | 144.8 |
| Ugurel | 53 | 97 | 22 | 97 | 0.2 | 63.2 |
| Caramuta (includes Danioti, is included in Edlund-Rose) | 12 | 32 | 9 | 32 | 2 | 177 |
| Elsas | Not tested | Not tested | 42 | 270 | 1 | 100 |
| Omholt | Not tested | Not tested | 21 | 73 | Unknown | Unknown |
| Demunter (codons 12 and 61 with x2 test) | Not tested | Not tested | 16 | 51 | 6 | 172 |
| Pouryazdanparast | 19 | 32 | 4 | 32 | 1 | 104 |
| Jacob | 112 | 206 | 66 | 160 | 1 | 48 |
| Brown | 23 + 20(metastatic) | 115 + 29(metastatic) | Not tested | Not tested | 2 | 144 |
| Lu (HR from IPD) | 106 | 395 | 29 | 395 | 3 | 229 |
| Akslen | 15 | 51 | 14 | 52 | 13 | 210 |

| | | | | | | |
|---|---|---|---|---|---|---|
| Menzies (Includes Long) | 143 | 301 | Not tested | Not tested | 1 | 60 |
| Turri | 1 | 32 | 7 | 32 | 3 | 94 |
| Takata | 9 | 21 | 3 | 11 | 1 | 148 |
| Casula | 11 | 12 | Not tested | Not tested | 15 | 55 |
| Kumar | 26 | 38 | 3 | 38 | 1 | 200 |

**Table 7: Summary of assessment of quality for the included studies.**

| Main Author | Year | Incomplete Outcome Data Risk | Selective Outcome Reporting Risk | Other bias Risk |
|---|---|---|---|---|
| van Elsas | 1996 | High Risk (Attrition) | High risk | High risk |
| Demunter | 2001 | Low risk | Low risk | Low risk |
| Omholt | 2002 | High Risk (Attrition) | Low risk | High risk |
| Omholt | 2003 | High Risk (Attrition) | High risk | High risk |
| Kumar | 2003 | Low Risk | Low risk | Low risk |
| Shinozaki | 2004 | High Risk (Exclusion) | High risk | High risk |
| Deichmann | 2004 | High Risk (Exclusion) | Low risk | High risk |
| Casula | 2004 | High Risk (Attrition) | High risk | High risk |
| Akslen | 2005 | Low risk | High risk | Low risk |
| Edlundh-Rose | 2006 | High Risk (Attrition) | High risk | High risk |
| Ugurel | 2007 | Low Risk | Low risk | Low risk |
| Takata | 2007 | Low Risk | High risk | Low risk |
| Janssen | 2008 | Low Risk | High risk | Low risk |
| Broekaert | 2010 | High Risk (Attrition) | Low risk | Low risk |
| Caramuta | 2010 | Low Risk | Low risk | Low risk |
| Devitt | 2011 | High Risk (Attrition) | High risk | High risk |
| Long | 2011 | Low Risk | Low risk | High risk |
| Jacob | 2011 | Low risk | Low risk | High risk |
| Pouryazdanparast | 2012 | High Risk (Attrition) | High risk | Low risk |
| Brown | 2012 | Low risk | Low risk | High risk |
| Lu | 2012 | Low risk | Low risk | Low risk |
| Menzies | 2012 | Low Risk | Low risk | High risk? |
| Turri | 2012 | Low Risk | Low risk | Low risk |

**Table 8: Assessment of blinding of histopathological assessment and incomplete outcome data risk for the included studies.**

| Main Author | Blinding of histopathologic assessment | Incomplete Outcome Data Risk | Incomplete Outcome Data Notes |
|---|---|---|---|
| Broekaert | Yes to clinical and genetic information of the specimens. | High Risk (Attrition) | They don't have the same data for all 365 cutaneous melanomas. BRAF status could not be obtained in 45 cases and NRAS or wild type status in 69 cases. Out of the 69 cases, 32 were wild type for BRAF, therefore 45 and 32 = 77 were the incomplete outcome data cases which agrees with 365-159 (BRAF)-51(NRAS)-78(WT). 45 and 37 are quite close and therefore the missing outcome data seem to be balanced. For their survival analysis they have data for only 257 cases and so they miss 108 cases. However, this has to do with analysing the tissue and probable has no effect to survival data analysis. Out of the 288 known mutation status they do not have survival data for 31 cases, which all have the BRAF mutation (159-128=31). Therefore, there is a high risk of incomplete outcome data bias. |
| Devitt | Due to the restrospective collection of survival data and genetic analysis, the histopathologic assessment was done previously and obviously was blinded to the above. | High Risk (Attrition) | 209 out of 249 had complete follow up history. They are missing 40 patients or about 20% of their initial cohort. |
| Shinozaki | Due to the restrospective collection of survival data and genetic analysis, the histopathologic assessment was done previously and obviously was blinded to the above. | High Risk (Exclusion) | 56 out of 59 had survival data. However, only patients with primary tumours were followed. Only DFS is mentioned. |
| Janssen | Due to the restrospective collection of survival data and genetic analysis, the histopathologic assessment was done previously and obviously was blinded to the above. | Low Risk. | Survival data are available for all choroidal melanomas but not for cilliary body. However, that should be OK for that just one group. |
| Omholt (only for sensitivity analysis as the data are included in the Edlundh-Rose) | Due to the restrospective collection of survival data and genetic analysis, the histopathologic assessment was done previously and obviously was blinded to the above. | High Risk (Attrition) | There is certainly missing data with regard to survival and censoring. |
| Edlundh-Rose (Includes Omholt 2003 and Caramuta 2010) | Due to the restrospective collection of survival data and genetic analysis, the histopathologic assessment was done previously and obviously was blinded to the above. | High Risk (Attrition) | We have no way of knowing how many patients were included in the survival analysis. |

| Deichmann | The histopathologic evaluation was done by one person. Although, it is not mentioned, he was probably blinded to the genetic analysis. | High Risk (Exclusion) | Survival analysis was not one of the outcomes of this article. Therefore, unavoidably there are missing data, mainly of the patients with no mutation. |
|---|---|---|---|
| Long | Not mentioned. Probably not done. | Low Risk. | They have some missing parameters data on only 42 patients. This does not affect survival analysis. |
| Ugurel | Yes due to study design. The histopathological grading came before the genetic analysis. | Low Risk. | Because this was a prospective study they do not have incomplete outcome data. |
| Caramuta (includes Danioti, is included in Edlund-Rose) | Due to the restrospective collection of survival data and genetic analysis, the histopathologic assessment was done previously and obviously was blinded to the above. | Low Risk. | Although, survival per BRAF or NRAS status was not the aim of this research, full data are available for 32 patients. |
| Elsas | Due to the restrospective collection of survival data and genetic analysis, the histopathologic assessment was done previously and obviously was blinded to the above. | High Risk (Attrition) | They have survival data only for 16+81=97 patients, almost one third of the total patients. |
| Omholt | Due to the restrospective collection of survival data and genetic analysis, the histopathologic assessment was done previously and obviously was blinded to the above. | High Risk (Attrition) | There is certainly missing data with regard to survival and censoring. |
| Demunter (codons 12 and 61 with x2 test) | Yes due to study design. The histopathological grading came before the genetic analysis. It is also mentioned in the article. | Low risk | Their aim was to compare survival of NRAS codon 18 patients and they include all available data with regard to that. |
| Pouryazdanparast | Due to the restrospective collection of survival data and genetic analysis, the histopathologic assessment was done previously and obviously was blinded to the above. | High Risk (Attrition) | There are 7 cases out of 40 that did not have follow up data available |
| Jacob | Due to the restrospective collection of survival data and genetic analysis, the histopathologic assessment was done previously and obviously was blinded to the above. | Low risk | They have very few missing patients from the initially selected. |
| Brown | Due to the restrospective collection of survival data and genetic analysis, the histopathologic assessment was done previously and obviously was blinded to the above. | Low risk | They investigated all those who could examine for BRAF, even though it was not the mprimary endpoint of their research. |

| | | | |
|---|---|---|---|
| Lu (HR from IPD) | Due to the restrospective collection of survival data and genetic analysis, the histopathologic assessment was done previously and obviously was blinded to the above. | Low risk | He has 37 missing patient data which is small number compared to his total size of 432 patients |
| Akslen | Due to the restrospective collection of survival data and genetic analysis, the histopathologic assessment was done previously and obviously was blinded to the above. | Low risk | They have only 6 out of the 57 patients that they did not have complete analysis of mutation status. |
| Menzies (Includes Long) | Not mentioned. Probably not done. | Low Risk. | They have some missing and excluded patients. However, this does not affect their analysis. |
| Turri | Due to the restrospective collection of survival data and genetic analysis, the histopathologic assessment was done previously and obviously was blinded to the above. | Low Risk. | They have no missing data |
| Takata | Due to the restrospective collection of survival data and genetic analysis, the histopathologic assessment was done previously and obviously was blinded to the above. | Low Risk. | The authors do not have data on 3 out of the 24 patients. |
| Casula | Due to the restrospective collection of survival data and genetic analysis, the histopathologic assessment was done previously and obviously was blinded to the above. | High Risk (Attrition) | Only a very small proportion of patients had tumour tissue analysis. |
| Kumar | Due to the restrospective collection of survival data and genetic analysis, the histopathologic assessment was done previously and obviously was blinded to the above. | Low Risk. | They have no missing data |

**Table 9: Assessment of selective outcome reporting and other bias risk for the selected studies.**

| Main Author | Selective Outcome Reporting Risk | Selective Outcome Reporting Notes | Other bias Risk | Other bias Notes (e.g. early stopping, baseline like gender and prognostic, study design, finance, fraudulence) |
|---|---|---|---|---|
| Broekaert | Low risk. | They never did survival analysis for NRAS. However, they do have all their analyses in the supplementary material. | Low risk. | Funding has been only from research centres. Nil else wrong was identified. |
| Devitt | High risk. | They do not report data on per clinical stage analysis, although they publish the survival curves. There are also missing p-values for the survival curves and numbers at risk for the overall survival after relapse. | High risk. | There is significant difference in the baseline characteristics as seen by p-values apart from sex. Most of their tumours are stages I and II that they already have better prognosis. They should have done analysis per stage. No financial connections were reported and the finance came gy a research centre. |
| Shinozaki | High risk. | They do not report the proportional hazards results. No numbers or p-value on the KM curve. | High risk. | There almost double number of males to females. Disproportionate amount of stage IV patients to other stages. No fraudulence or suspicious finance. |
| Janssen | High risk. | There is no p-value for the logrank test. There is no survival analysis according to DFS or OS, although data are available. | Low risk. | Finance was by research organisations. Nil else wrong was identified. |
| Omholt (only for sensitivity analysis as the data are included in the Edlundh-Rose) | High risk. | They do not report separate p-values for comparison between two groups of BRAF, NRAS and WT. | High risk. | The survival analysis is inappropriate as it does not take account censoring. There are no financial issues. They included only patients whose disease had progressed to metastatic and therefore they seem to have a much higher BRAF mutation to WT proportion than other articles. They compare mutation status to WT free of NRAS or BRAF. |

| | | | | |
|---|---|---|---|---|
| Edlundh-Rose (Includes Omholt 2003 and Caramuta 2010) | High risk. | They do not report separate p-values for comparison between two groups of BRAF, NRAS and WT. | High risk. | No financial issues. The survival analysis is appropriate but for such a long follow up (25 years) overall survival is not appropriate. DFS or MSS would have been more appropriate. Also the proportion of mutations may have changed in the population during all that time. |
| Deichmann | Low risk. | Survival analysis was not the aim of this article, although there are data available. | High risk. | Finance was from a pharmaceutical company (Roche). Age and gender were not examined as baseline variables. |
| Long | Low risk. | They report everything. | High risk. | Although there was no mention of funding, some of the authors have financial relationship with GlaxoSmithKline or Roche. There is also a major study design flaw where they mix the tissues for genetic analysis (primary, locoregional and metastatic) |
| Ugurel | Low risk. | They do not report univariate Cox hazards, but that was probably thought as secondary. They do not report the p value between each group: BRAF, NRAS, WT. However, it seems from the survival curves that BRAF and WT are about the same and only the presence of NRAS makes any difference in the survival. | Low risk. | They do a separate analysis for stage IV so as to avoid differences on baseline characteristics. The authors have no funding or other support. They declare no competing interest. |
| Caramuta (includes Danioti, is included in Edlund-Rose) | Low risk. | This is not applicable as the survival per BRAF was not the aim of this research article. Also, we are using the IPD data. | Low risk. | Finance was by research organisations. Nil else wrong was identified. |
| Elsas | High risk. | They do not show the overall survival, although they say it was not significant. DFS and MSS would be more important and are not reported. | High risk. | Finance was from research centres. There is great heterogeneity in the specimens, e.g. more males, many countries, which is not explored fully in the survival analysis. |
| Omholt | Low risk | There does not seem to exist selective outcome reporting as they present only the prognostic value of NRAS. | High risk. | The survival analysis is inappropriate as it does not take account censoring. There are no financial issues. They included only patients whose disease had progressed to metastatic. They compare mutation status to WT including BRAF. |
| Demunter (codons 12 and 61 with x2 test) | Low risk | They also include prognostic data on mutations 61 and 12. | Low risk. | They did the survival analysis using log-rank tests (appropriately). No finance issues. They included patients whose metastasis were not shown yet (stages I and II) to identify the progress of the disease. |
| Pouryazdanparast | High risk. | Although the data are available they do not do survival analysis, even for the group they are analysing. | Low risk. | Most of the funding is from non-pharmaceutical foundations, although there is some support from a pharmaceutical company. Their study design is appropriate but for the missing survival analysis, which is already mentioned elsewhere. No other bias noted. |

| | | | | |
|---|---|---|---|---|
| Jacob | Low risk | They do a very thorough statistical analysis. | High risk. | The funding was from research organizations. Good study design. Multivariate analysis using Cox. Most of the authors have relationship with pharmaceutical companies, which is declared. Moreover their findings are in aggreement with that of other researchers. They do not mention where they took their specimens from (primary or metastatic, tissue or cells and how they were fixated). |
| Brown | Low risk | They do a good statistical analysis and report all of OS, DFS and MSS. | High risk. | There is no suspicious funding. They do search though for only one BRAF mutation (V600E) and their percentage in primaries is less than expected, due to possible underdetection. |
| Lu (HR from IPD) | Low risk | They do report everything they have and they publish their full IPD data. | Low risk. | No suspicious funding reported. They searched for all the mutations. They could have searched for DFS and MSS, but in any case, they report everything else. |
| Akslen | High risk. | They do not report the statistical results of the survival analysis. | Low risk. | No suspicious funding. They could have done more with the data they had but this was mentioned in the selective outcome reporting. |
| Menzies (Includes Long) | Low risk. | They report everything. | High risk? | Many members of this article have relationships with pharmaceutical companies. However the grant comes from research organizations. There is also a major study design flaw where they mix the tissues for genetic analysis (primary, locoregional and metastatic) |
| Turri | Low risk | They do give IPD data, which makes further research easier. | Low risk. | Funding is mentioned. No other risk is identified for the type of research they did. |
| Takata | High risk. | The authors do not report the survival analysis on their patients. However, they kindly offered their IPD data. | Low risk. | Funding is by government agencies. No competing interests. Other than the selective reporting none other risk is identified. |
| Casula | High risk. | The authors have provided the data on 23 patients. However, no statistics are given in the article for the presumed survival analysis of 358 patients. | High risk. | The authors report no conflict of interest. Support is by government organizations. However, survival analysis cannot be based on x2. Germline analysis proves only that the mutations are somatic and nothing else on melanoma. |
| Kumar | Low risk | They report almost everything. They do not report survival on NRAS, probably due to small numbers. | Low risk. | There is no mention of funding. However due to the significant retrospective range of the analysis (1988 to 1996) it is unlikely to be spurious. Survival analysis is appropriate and IPD data are available. |

### *Standard statistical analysis*

The primary endpoint of the study was OS, followed by MSS and DFS and then by the secondary endpoints of the various subgroup analysis suggested. An IPD meta-analysis was also performed.

### Data synthesis

We used the *HR* and corresponding CI extracted from each study to assess between-study heterogeneity using the Q statistic and inconsistency using the $I^2$ statistic. Heterogeneity was considered significant at the $p<0.1$ level. Summary *HRs* with their 95% CI were calculated using an inverse variance method. HRs greater than 1 are suggestive of increased risk of death for BRAF or NRAS mutated tumours. We fitted a random-effects model since between-study heterogeneity was anticipated. We also fitted fixed-effects model for comparison. For the meta-analysis, OS, DFS and MSS were defined as the primary outcomes and subgroup analyses as secondary outcomes. We assessed whether there was a differential magnitude of effects in large versus small studies, commonly referred to as “publication bias”, using the Egger and Peter regression tests. We also checked for publication bias using funnel plots, radial plots and funnel plots with the trim and fill method.

To explore potential sources of between study heterogeneity we performed subgroup analyses based on the following factors: locality of the samples, design of the study, histopathological subtype, male versus female, primary versus metastatic melanoma on the prognostic value of BRAF and NRAS somatic mutations. Although, it was the desire of the authors to investigate more sources, this was not possible due to the small number of studies and the fact that not all of them presented available data on all the possible confounders. For the same

reason all the analysis is univariate and a test for interaction for comparisons between subgroups was not possible. However, this was possible for some variables in the IPD data one stage meta-analysis.

Statistical analyses were conducted with the R open-source freeware statistical package (version 2.15.2). P-values for all comparisons were two-tailed and statistical significance was defined as $p<0.05$ for all tests except those for heterogeneity.

**Table 10: Key to calculated methods for extracted data**

| | |
|---|---|
| 1 | Report presents observed and expected events on research and control |
| 2a | Report presents HR and V |
| 2b | Report presents HR and O-E |
| 2c | Report presents O-E and V |
| 3 | Report presents HR and CIs |
| 4 | Report presents HR and events in each arm and randomisation ratio is 1:1 |
| 5 | Report presents HR and total events and randomisation ratio is 1:1 |
| 6 | Report presents HR, total events and the no.s analysed on each arm and randomisation ratio need not be 1:1 |
| 7 | Report presents p-value and events on each arm and randomisation ratio is 1:1 |
| 8 | Report presents p-value and total events and randomisation ratio is 1:1 |
| 9 | Report presents p-value, total events & no.s analysed on each arm and randomisation ratio need not be 1:1 |
| 10 | Data from curve read where wished and assuming constant censoring |
| 11 | Data from curve with numbers at risk given |

**Overall Survival (OS), Melanoma Specific Survival (MSS) and combined OS and MSS of patients with BRAF and wild type (WT) mutations**

We start our analysis with our primary outcome, which is Overall Survival (OS). Initially 10 studies were included (see table). We have also analysed Melanoma Specific Survival (MSS). However, we are unsure as to what kind of survival did the authors that refer to OS have analysed. Long et. al. refer to OS. However, Menzies et. al. speaks about MSS. Only 3 studies out of the 23 that were finally included for analysis of OS and DFS (Disease Free Survival) of BRAF and NRAS mutations report both OS and MSS.

Below are three tables with information on the studies included and which we used for our calculations. This information was first extracted from the relevant article and then was used to calculate the *HRs*. HRs more than 1 are suggestive of increased risk of death for the patients carrying the mutation whereas lower than 1 are suggestive of less risk. When more than one way of calculations was available, then the range of *HRs* and variance is given. For a key to the method, please see the Table 10 and read the section on the extraction of data methods. The method chosen was either the only one or the most reliable available with regard to the data published. What we mean by that, is that although more than one ways may be available, the authors did present their data in only one way (usually survival curves or Cox *HRs*) and we take those data as the most reliable. In many cases we could extract information from other sections of the article/research in question but we cannot possibly know whether those data correspond to the truth or can be used for survival analysis. In most cases though, there were only small differences, affecting the variance more than the *HR,* as you can see from the tables below. We already know that there is significant heterogeneity between the various studies due to many reasons. They

come from different continents, they used different methods to search for BRAF mutations, some searched more exons than the exon 15, some used metastatic, others primary and some both type of tissues for analysis. Some enlisted only metastatic patients and some researched only certain types of melanoma like mucosal, ocular or nodular. For these reasons we wil consider only the random-effects analysis and we will used the fixed-effects analysis results only for discussion or comparison.

**Table 11: Information on included studies with BRAF for OS, MSS, DFS analysis - part 1.**

| Author | Year | Continent | Countries | data_from | only_met_patients | Specimen_Type | fixation |
|---|---|---|---|---|---|---|---|
| **Broekaert** | 2010 | NorthA_Eu | USA_Eu | KM | no | pri_cut | formalin |
| **Devitt** | 2011 | Oceania | Australia | COX_ARTICLE | no | pri_cut | formalin |
| **Shinozaki** | 2004 | NorthA | USA | KM | no | pri_cut | formalin |
| **Janssen** | 2008 | Europe | UK | KM | no | occular | formalin |
| **Omholt** | 2003 | Europe | Sweden | PVALUE_X2 | yes | pri_cut | formalin |
| **Edlundh-Rose** | 2006 | Europe | Sweden | KM | yes | pri_met | form_froz |
| **Deichmann** | 2004 | Europe | Germany | PVALUE_BIN_LOG_REG | no | pri_cut | formalin |
| **Long** | 2011 | Oceania | Australia | KM | yes | pri_met | formalin |
| **Ugurel** | 2007 | Europe | Germany | IPD | yes | metastatic | frozen |
| **Caramuta** | 2010 | Europe | Sweden | IPD | no | metastatic | formalin |
| **Pouryazdanparast** | 2012 | NorthA | USA | IPD | no | pri_cut | formalin |
| **Jacob** | 2011 | NorthA | USA | KM | yes | metastatic | NA |
| **Brown** | 2012 | Europe | UK | KM | no | pri_met | formalin |
| **Lu** | 2012 | Asia | China | IPD | no | pri_cut | formalin |
| **Akslen** | 2005 | Europe | Norway | IPD | no | pri_cut | formalin |
| **Menzies** | 2012 | Oceania | Australia | KM | yes | pri_met | formalin |
| **Takata** | 2007 | Asia | Japan | IPD | no | pri_cut | formalin |
| **Casula** | 2004 | Europe | Italy | IPD | no | pri_met | NA |
| **Kumar** | 2003 | Europe | Sweden | IPD | yes | pri_met | frozen |

**Table 12: Information on included studies with BRAF for OS, MSS, DFS analysis - part 2.**

| Author | Year | time_from | design | braf_exons | blinding | IOR | SOR | other_risk |
|---|---|---|---|---|---|---|---|---|
| **Broekaert** | 2010 | primary | Retrospective | 15 | Y | High | Low | Low |
| **Devitt** | 2011 | primary | Prospective | 15 | Y | High | High | High |
| **Shinozaki** | 2004 | primary | Prospective | 11_15 | Y | High | High | High |
| **Janssen** | 2008 | primary | Retrospective | 15 | Y | Low | High | Low |
| **Omholt** | 2003 | primary | Retrospective | 11_15 | Y | High | High | High |
| **Edlundh-Rose** | 2006 | primary | Retrospective | 11_15 | Y | High | High | High |
| **Deichmann** | 2004 | primary | Retrospective | 15 | Y | High | Low | High |
| **Long** | 2011 | primary | Prospective | 15 | N | Low | Low | High |
| **Ugurel** | 2007 | metastatic | Prospective | 11_15 | Y | Low | Low | Low |
| **Caramuta** | 2010 | metastatic | Retrospective | 15 | Y | Low | Low | Low |
| **Pouryazdanparast** | 2012 | primary | Retrospective | 15 | Y | High | High | Low |
| **Jacob** | 2011 | metastatic | Retrospective | 15 | Y | Low | Low | High |
| **Brown** | 2012 | primary | Retrospective | 15 | Y | Low | Low | High |
| **Lu** | 2012 | primary | Retrospective | 11_15 | Y | Low | Low | Low |
| **Akslen** | 2005 | primary | Retrospective | 15 | Y | Low | High | Low |
| **Menzies** | 2012 | primary | Prospective | 15 | N | Low | Low | High |
| **Takata** | 2007 | primary | Retrospective | 15 | Y | Low | High | Low |
| **Casula** | 2004 | primary | Retrospective | 3_15 | Y | High | High | High |
| **Kumar** | 2003 | primary | Retrospective | 1_11_15 | Y | Low | Low | Low |

**Table 13: Information on included studies with BRAF for OS, MSS, DFS analysis - part 3.**

| Author | Year | braf_n | braf_n_wt | braf_n_total | hr_dfs_braf | hr_os_braf | hr_mss_braf | hr_osmss_braf |
|---|---|---|---|---|---|---|---|---|
| Broekaert | 2010 | 159 | 129 | 288 | 1.12 | 1.15 | | 1.15 |
| Devitt | 2011 | 112 | 137 | 249 | 1.82 | 1.22 | 1.64 | 1.22 |
| Shinozaki | 2004 | 18 | 41 | 59 | 2.54 | | | |
| Janssen | 2008 | 11 | 19 | 30 | | | 1.74 | 1.74 |
| Omholt | 2003 | 42 | 29 | 71 | | 1.06 | | 1.06 |
| Edlundh-Rose | 2006 | 120 | 99 | 219 | | 0.74 | | 0.74 |
| Deichmann | 2004 | 19 | 31 | 50 | 0.69 | | | |
| Long | 2011 | 95 | 102 | 197 | 1.19 | 1.99 | | 1.99 |
| Ugurel | 2007 | 53 | 44 | 97 | | 1.63 | | 1.63 |
| Caramuta | 2010 | 12 | 20 | 32 | | 0.94 | 1.35 | 0.94 |
| Pouryazdanparast | 2012 | 19 | 13 | 32 | 0.51 | | 0.25 | 0.25 |
| Jacob | 2011 | 112 | 94 | 206 | | 1.31 | | 1.31 |
| Brown | 2012 | 43 | 101 | 144 | 2.62 | 4.41 | 7.59 | 4.41 |
| Lu | 2012 | 106 | 289 | 395 | | 1.43 | | 1.43 |
| Akslen | 2005 | 15 | 36 | 51 | | 1.65 | 1.95 | 1.65 |
| Menzies | 2012 | 143 | 158 | 301 | 0.98 | 1.44 | | 1.44 |
| Takata | 2007 | 9 | 12 | 21 | 1.60 | 0.48 | | 0.48 |
| Casula | 2004 | 11 | 1 | 12 | 1.30 | 0.86 | | 0.86 |
| Kumar | 2003 | 26 | 12 | 38 | 0.56 | | | |

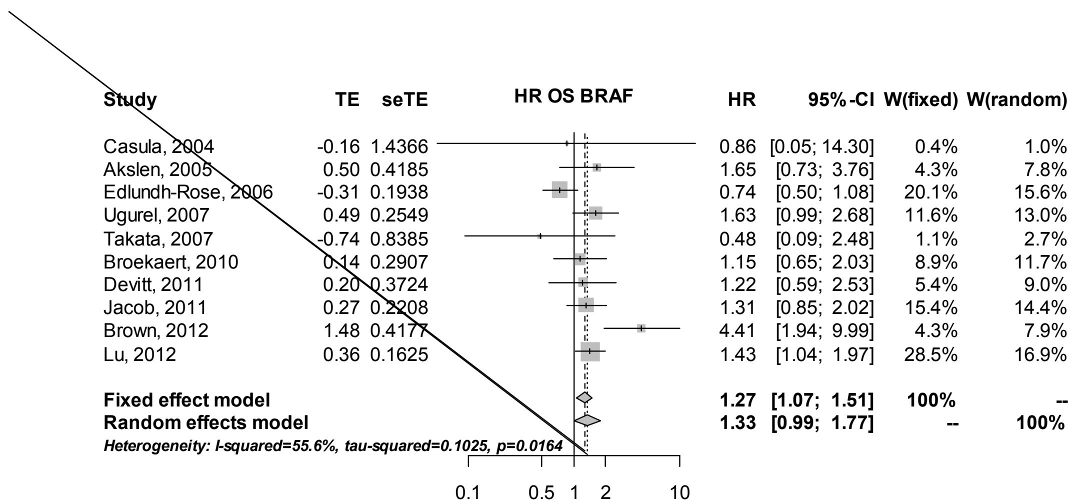
We start our analysis with testing first the 10 studies for OS then the 6 studies for MSS and finally we combine all studies having either OS or MSS. For the two studies that measure both (by Devitt and Brown) we pick the OS data for analysis. OS underestimates the survival because the subjects may have died from other causes than their melanoma disease. However, it is less likely to show a difference when there is none, unless more subjects are dying from other causes in only one of the two groups under question.

The heterogeneity between the studies is significant as seen by $I^2$ of 55.6% and Cohrane's Q p-value of 0.0164. Our *HR* is statistically significant for a fixed-effects model and borderline significant for a random-effects model. Of course with such a significant heterogeneity, only a random-effects model is appropriate, which shows a 33% increase in the risk of death for patients with melanoma and the BRAF mutation compared to wild-type patients (HR=1.33, p-value=0.0544).:

```
                     HR          95%-CI %W(fixed) %W(random)
Casula, 2004       0.86  [0.05; 14.30]      0.36       1.01
Akslen, 2005       1.65  [0.73;  3.76]      4.30       7.85
Edlundh-Rose, 2006 0.74  [0.50;  1.08]     20.05      15.56
Ugurel, 2007       1.63  [0.99;  2.68]     11.59      13.01
Takata, 2007       0.48  [0.09;  2.48]      1.07       2.71
Broekaert, 2010    1.15  [0.65;  2.03]      8.91      11.65
Devitt, 2011       1.22  [0.59;  2.53]      5.43       9.04
Jacob, 2011        1.31  [0.85;  2.02]     15.44      14.41
Brown, 2012        4.41  [1.94;  9.99]      4.32       7.87
Lu, 2012           1.43  [1.04;  1.97]     28.52      16.90

Number of studies combined: k=10

                       HR         95%-CI    z  p.value
Fixed effect model   1.27  [1.07; 1.51] 2.76   0.0058
Random effects model 1.33  [0.99; 1.77] 1.92   0.0544

Quantifying heterogeneity:
tau^2 = 0.1025; H = 1.5 [1.05; 2.14]; I^2 = 55.6% [9.7%; 78.1%]
```

```
Test of heterogeneity:
     Q d.f.  p.value
 20.25    9   0.0164

Details on meta-analytical method:
- Inverse variance method
- DerSimonian-Laird estimator for tau^2
```

The forest plot shows the *HRs*, CIs and weights for all the studies and the summary effect.




The Egger's test is not suggestive of bias but the Peter's test which the most appropriate is highly statistically significant of that.

## Egger's test:

```
Review:     HR_OS for BRAF

        Linear regression test of funnel plot asymmetry

data:  brafos.meta
t = 0.3297, df = 8, p-value = 0.7501
alternative hypothesis: asymmetry in funnel plot
sample estimates:
     bias   se.bias     slope
0.3670601 1.1133972 0.1494492
```

**Peters test:**

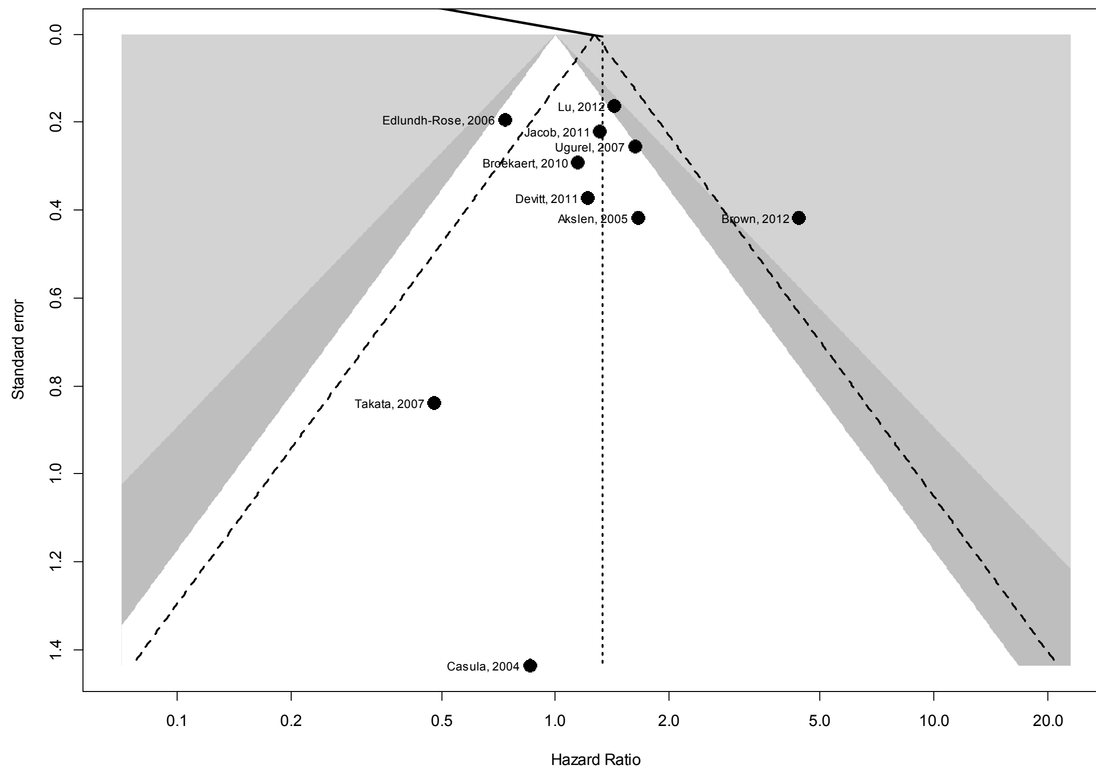
```
Regression Test for Funnel Plot Asymmetry

model:     mixed-effects meta-regression model
predictor: inverse of the total sample size

z = -3.0444, p = 0.0023
```

We continue with an influential analysis, where we exclude the studies one by one. We notice that when we exclude the study by Edlundh-Rose et al. (2006), or Brown et al. (2012) there seems to be little heterogeneity. That is understandable due to the opposite results compared to the other articles for the first study and the big difference in effect for the second. We also see that our analysis is not entirely robust as omitting certain studies renders the results statistically non-significant. That also shows the need for a meta-analysis and a large number of subjects to be included so that significant results are found.

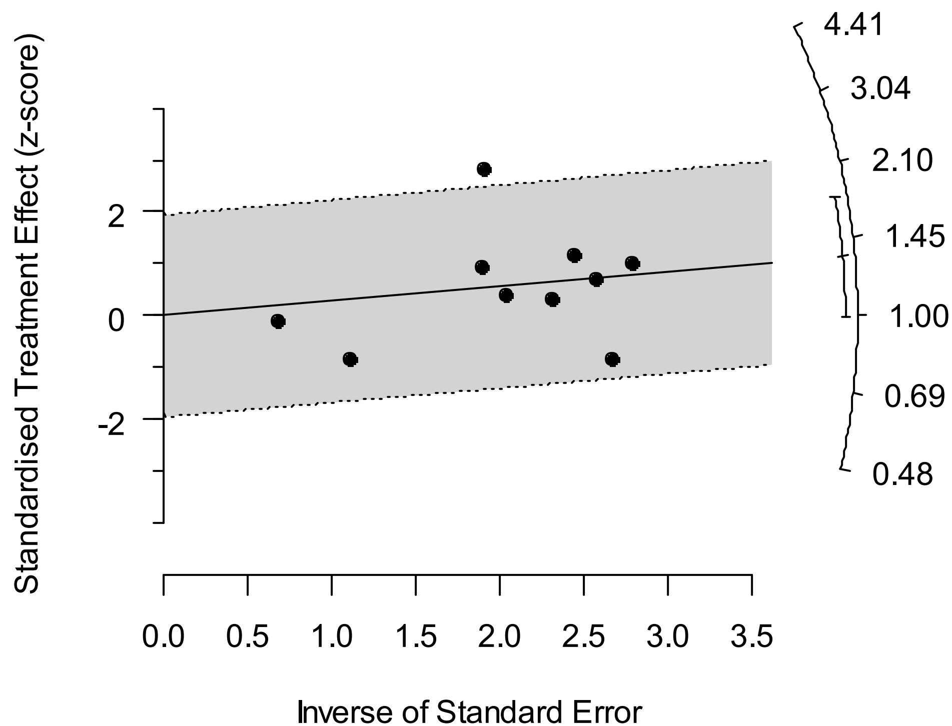
```
Influential analysis (Random effects model)

                                HR           95%-CI  p.value    tau^2
Omitting Casula, 2004        1.3366  [0.9919; 1.8011]   0.0566   0.1115
Omitting Akslen, 2005        1.3058  [0.9559; 1.7840]   0.0937   0.1146
Omitting Edlundh-Rose, 2006  1.4699  [1.1607; 1.8615]   0.0014   0.0278
Omitting Ugurel, 2007        1.2930  [0.9340; 1.7898]   0.1215   0.1199
Omitting Takata, 2007        1.3659  [1.0203; 1.8286]   0.0362   0.1007
Omitting Broekaert, 2010     1.3596  [0.9802; 1.8859]   0.0657   0.1251
Omitting Devitt, 2011        1.3440  [0.9770; 1.8488]   0.0692   0.1204
Omitting Jacob, 2011         1.3387  [0.9511; 1.8840]   0.0944   0.1377
Omitting Brown, 2012         1.2012  [0.9624; 1.4994]   0.105    0.0289
Omitting Lu, 2012            1.3185  [0.9263; 1.8766]   0.1248   0.1476

Pooled estimate              1.3284  [0.9947; 1.7742]   0.0544   0.1025
                                I^2
Omitting Casula, 2004          60.3%
Omitting Akslen, 2005          59.7%
Omitting Edlundh-Rose, 2006    22.6%
Omitting Ugurel, 2007          58.3%
```

```
Omitting Takata, 2007          57.6%
Omitting Broekaert, 2010       60.2%
Omitting Devitt, 2011          60.5%
Omitting Jacob, 2011           60.5%
Omitting Brown, 2012           27.2%
Omitting Lu, 2012              58.9%

Pooled estimate                55.6%

Details on meta-analytical method:
- Inverse variance method
- DerSimonian-Laird estimator for tau^2
```

In the following contour enhanced funnel plot we see the two studies (by Edludh-Rose et al. and Brown et al.) that increase heterogeneity by being outside the funnel plot lines. We may also be missing some small statistically significant studies with big positive HRs. That could be due to heterogeneity and not necessarily publication bias.



The same results are shown by the radial plot below where one study in not included within the 95% shaded area.




By a trim and fill analysis we see that although we have significant heterogeneity, there doesn't seem to be any significant publication bias and therefore no studies are added. That gives us more certainty that we have included all the available studies, even though the funnel plot is not entirely symmetrical. It seems that we are lacking some small studies with a HR>1 which may have pushed our results to even more statistical significance:

| | HR | 95%-CI | %W(fixed) | %W(random) |
|---|---|---|---|---|
| Broekaert, 2010 | 1.1470 | [0.6489; 2.0276] | 8.91 | 11.65 |
| Devitt, 2011 | 1.2200 | [0.5880; 2.5313] | 5.43 | 9.04 |
| Edlundh-Rose, 2006 | 0.7364 | [0.5037; 1.0766] | 20.05 | 15.56 |
| Ugurel, 2007 | 1.6256 | [0.9864; 2.6791] | 11.59 | 13.01 |
| Jacob, 2011 | 1.3100 | [0.8498; 2.0195] | 15.44 | 14.41 |

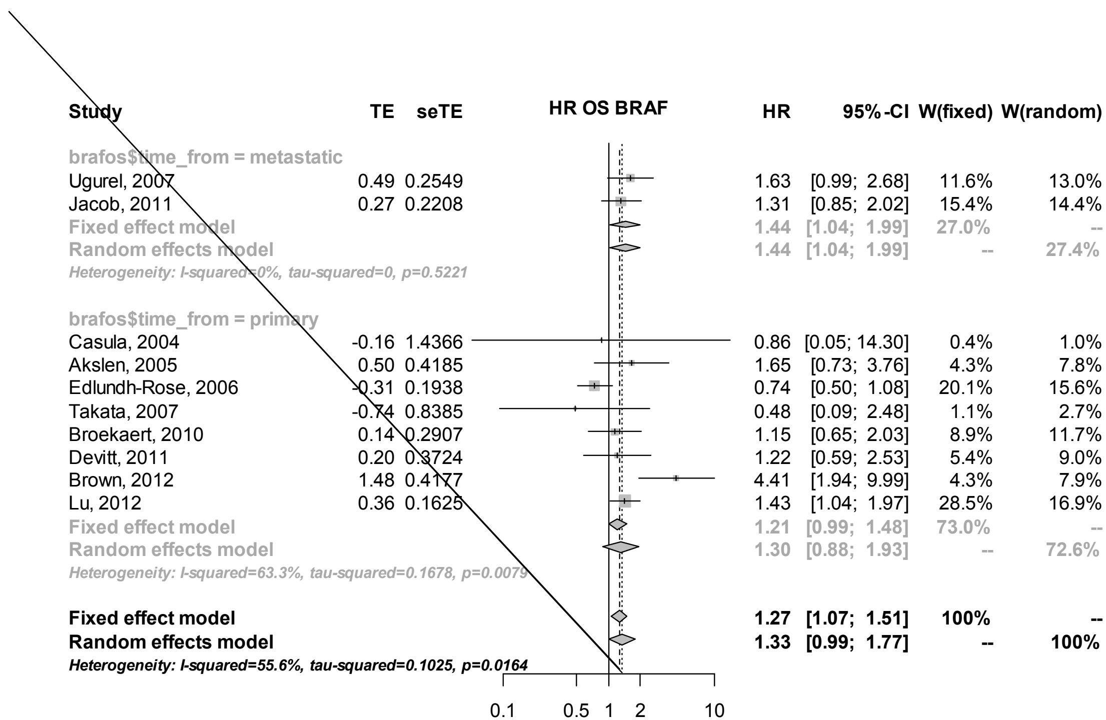
```
Brown, 2012         4.4058  [1.9431;  9.9898]      4.32       7.87
Lu, 2012            1.4329  [1.0421;  1.9703]     28.52      16.90
Akslen, 2005        1.6547  [0.7286;  3.7578]      4.30       7.85
Takata, 2007        0.4790  [0.0926;  2.4779]      1.07       2.71
Casula, 2004        0.8563  [0.0513; 14.3046]      0.36       1.01

Number of studies combined: k=10 (with 0 added studies)

                        HR            95%-CI      z  p.value
Fixed effect model   1.2706  [1.0718; 1.5061] 2.7595   0.0058
Random effects model 1.3284  [0.9947; 1.7742] 1.9238   0.0544

Quantifying heterogeneity:
tau^2 = 0.1025; H = 1.5 [1.05; 2.14]; I^2 = 55.6% [9.7%; 78.1%]

Test of heterogeneity:
     Q d.f.  p.value
 20.25    9   0.0164

Details on meta-analytical method:
- Inverse variance method
- DerSimonian-Laird estimator for tau^2
- Trim-and-fill method to adjust for funnel plot asymmetry
```

By doing cumulative meta-analysis we notice that the study by Lu was the one to "push" the analysis towards statistically significant results, even though they are borderline.

```
Cumulative meta-analysis (Random effects model)

                                     HR             95%-CI  p.value    tau^2
Adding Casula, 2004 (k=1)         0.8563  [0.0513; 14.3046]   0.914    0
Adding Akslen, 2005 (k=2)         1.5716  [0.7150;  3.4542]   0.2605   0
Adding Edlundh-Rose, 2006 (k=3)   0.9631  [0.5333;  1.7394]   0.9008   0.1058
Adding Ugurel, 2007 (k=4)         1.1713  [0.6785;  2.0219]   0.5703   0.161
Adding Takata, 2007 (k=5)         1.0886  [0.6562;  1.8059]   0.7424   0.1451
Adding Broekaert, 2010 (k=6)      1.0982  [0.7539;  1.5999]   0.6254   0.08
Adding Devitt, 2011 (k=7)         1.1077  [0.8090;  1.5168]   0.5235   0.0507
Adding Jacob, 2011 (k=8)          1.1407  [0.8818;  1.4757]   0.3162   0.0324
Adding Brown, 2012 (k=9)          1.3185  [0.9263;  1.8766]   0.1248   0.1476
```

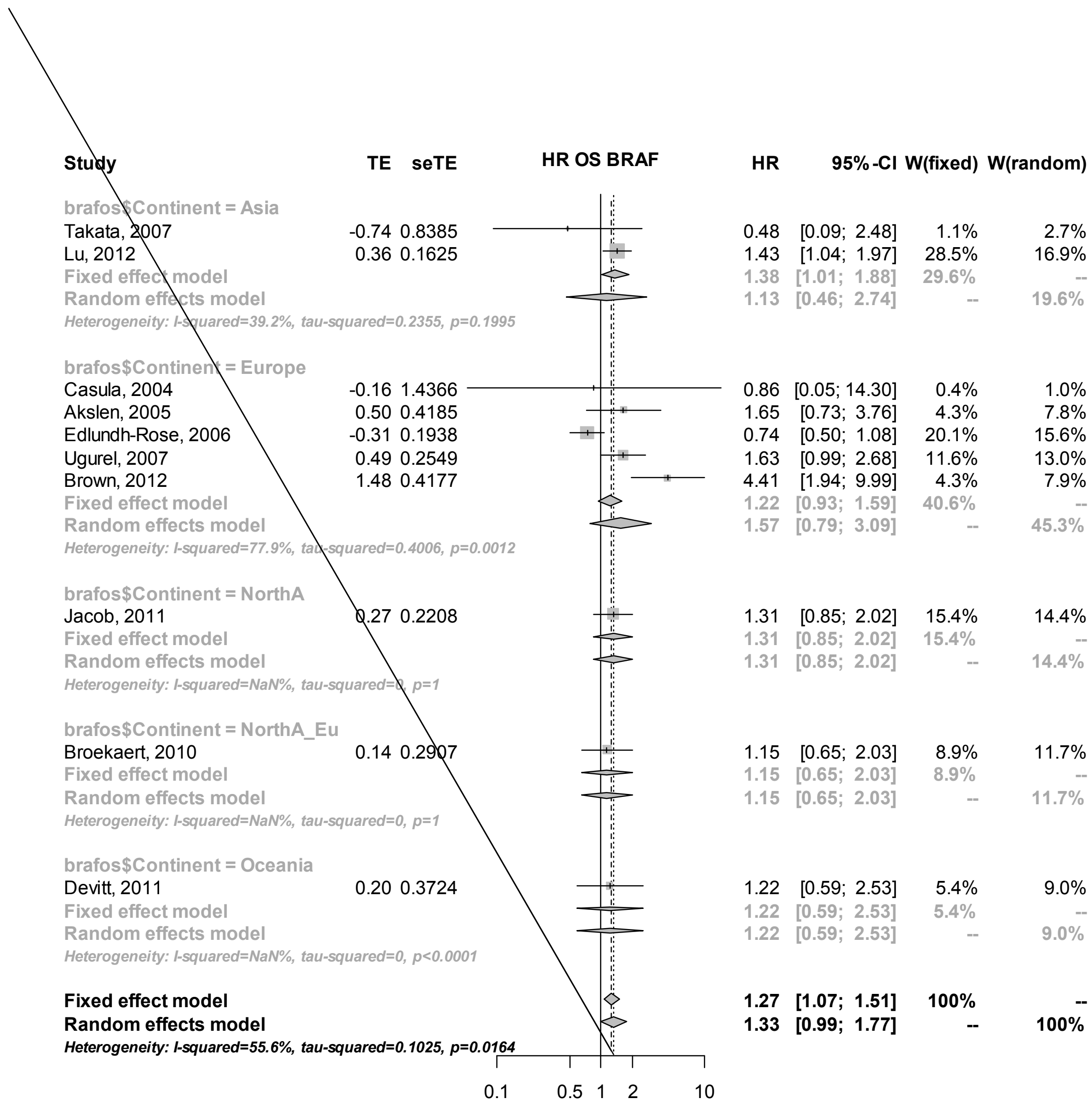
```
Adding Lu, 2012 (k=10)            1.3284  [0.9947;  1.7742]   0.0544   0.1025

Pooled estimate                   1.3284  [0.9947;  1.7742]   0.0544   0.1025
                                      I^2
Adding Casula, 2004 (k=1)
Adding Akslen, 2005 (k=2)              0.0%
Adding Edlundh-Rose, 2006 (k=3)       35.1%
Adding Ugurel, 2007 (k=4)             60.0%
Adding Takata, 2007 (k=5)             52.0%
Adding Broekaert, 2010 (k=6)          40.9%
Adding Devitt, 2011 (k=7)             30.4%
Adding Jacob, 2011 (k=8)              24.8%
Adding Brown, 2012 (k=9)              58.9%
Adding Lu, 2012 (k=10)                55.6%

Pooled estimate                       55.6%

Details on meta-analytical method:
- Inverse variance method
- DerSimonian-Laird estimator for tau^2
```

We will now try to identify the heterogeneity in our included studies. One factor is the time from where the OS is measured. Some studies measure OS from the time of biopsy of metastatic tumour whereas the usual is from the time of biopsy of primary tumour. Of course that is not always wrong as there will be cases where the primary diagnosis was done at the same time as the metastasis. However, that also suggests that these studies include only subjects with metastatic stages of melanoma (IIIC and IV). We prefer to present the forest plot of the subgroup analysis as is easier to read and more appealing to the eye of the reader.

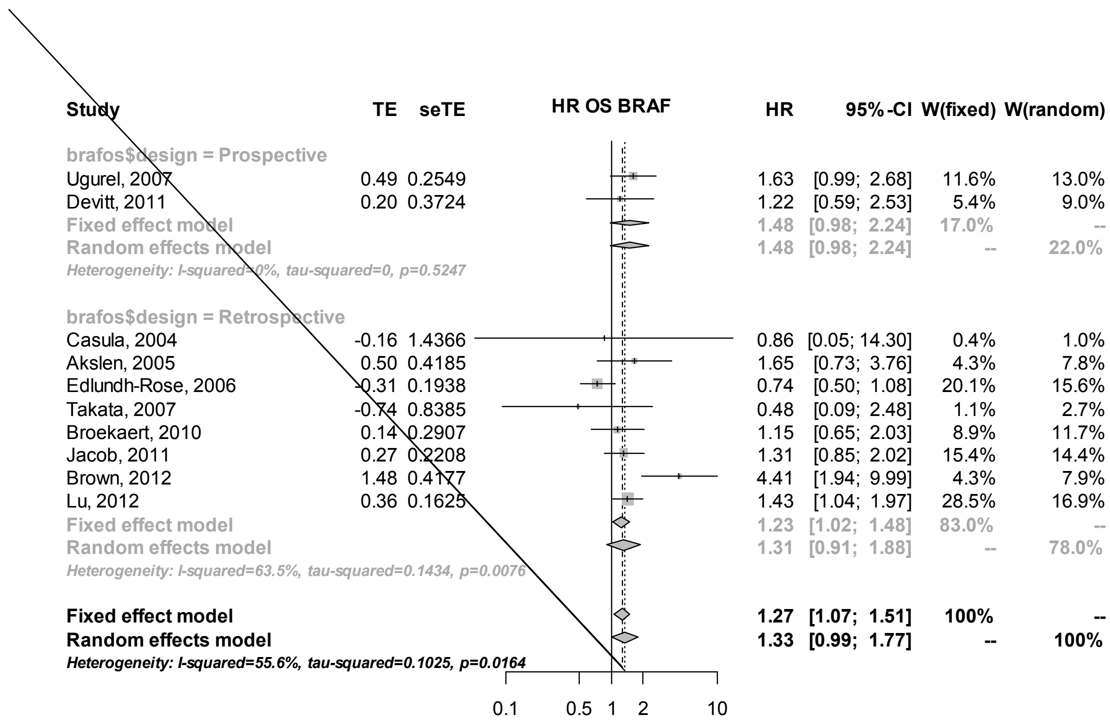



We notice that no heterogeneity is found for the studies, who measure OS from the time of metastasis, but they are only 2 and this of course is not entirely accurate. For the rest of the studies there is still significant heterogeneity between them as seen by a p-value of 0.0079. For the studies that measure OS from the time of metastatic diagnosis the HR is 1.04 under the fixed effect analysis.

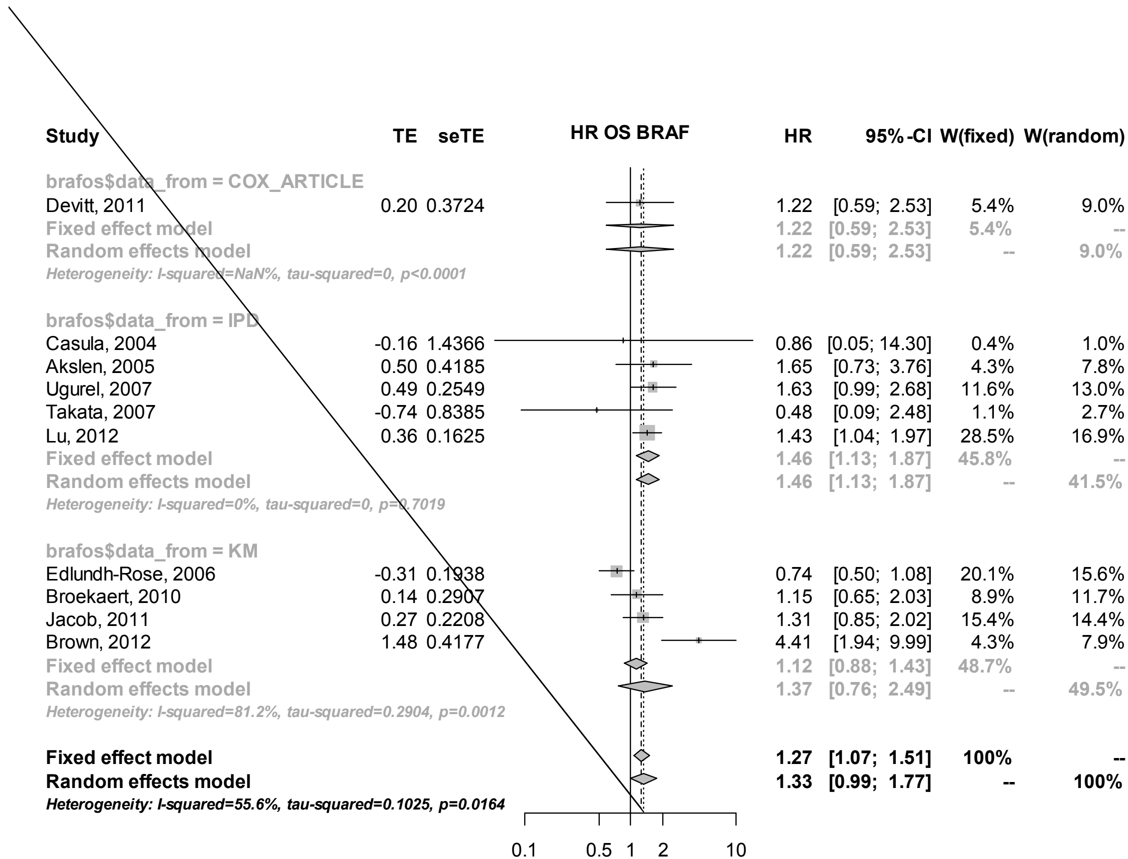
We continue with subgroup analysis based on the continent where only studies from Asia give statistically significant results and only under fixed-effects model, with HR=1.38.



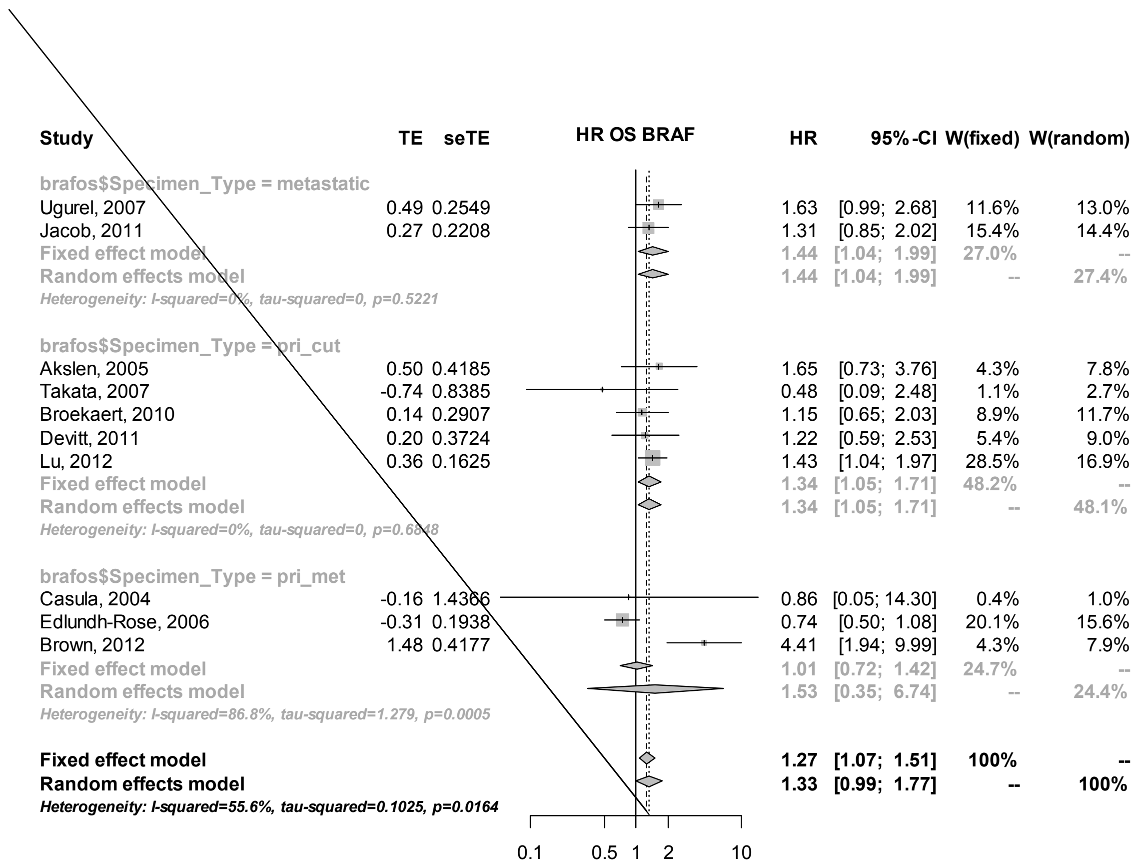
Subgroup analysis by design of the study does not reveal any significant results.



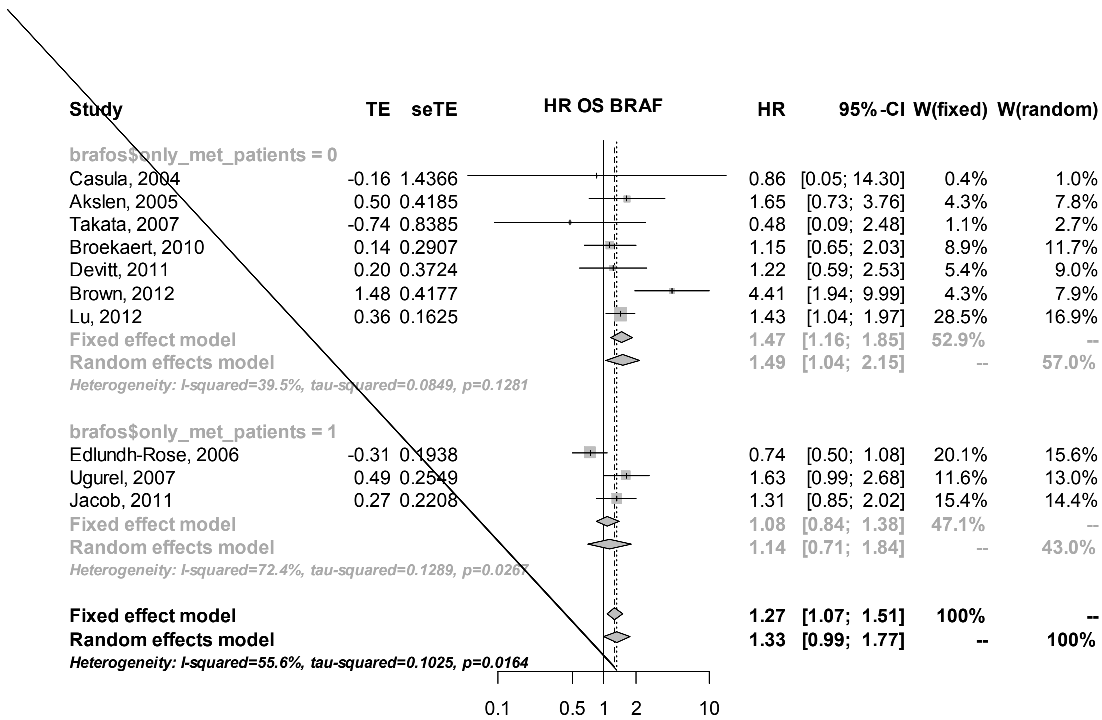
Subgroup analysis based on the way the data were extracted show statistically significant results for the data extracted through IPD analysis with a HR of 1.46.



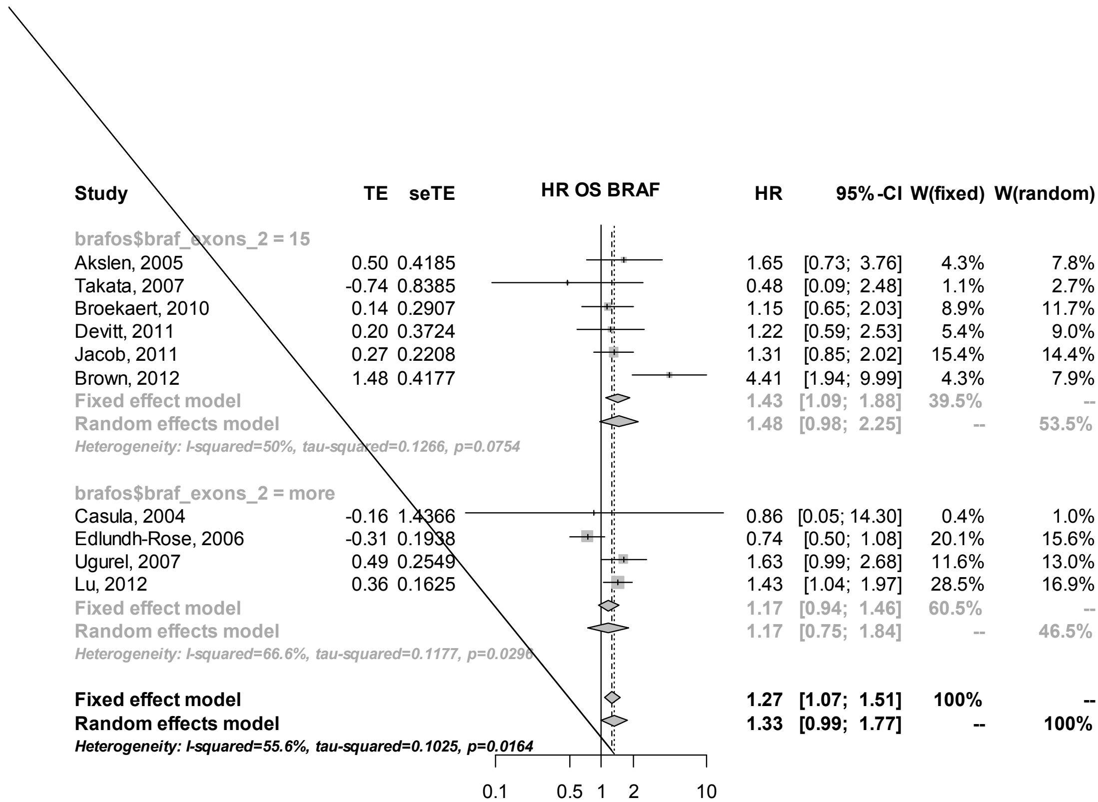

We get interesting results when we group studies based on whether they got their data by analysing only primary melanoma tumours, only metastatic melanoma tumours or a combination of both. We see that the most heterogenous group is the one consting of the combination of primary and metastatic tumours. Although research has shown that there may not be big difference between the two, however this may not necessarily be true. Many researchers have found a greater percentage of BRAF mutation in metastatic than in primary tumours and therefore the two types of tissue may reflect different stages in the pathophysiology of BRAF mutated melanoma tumours.



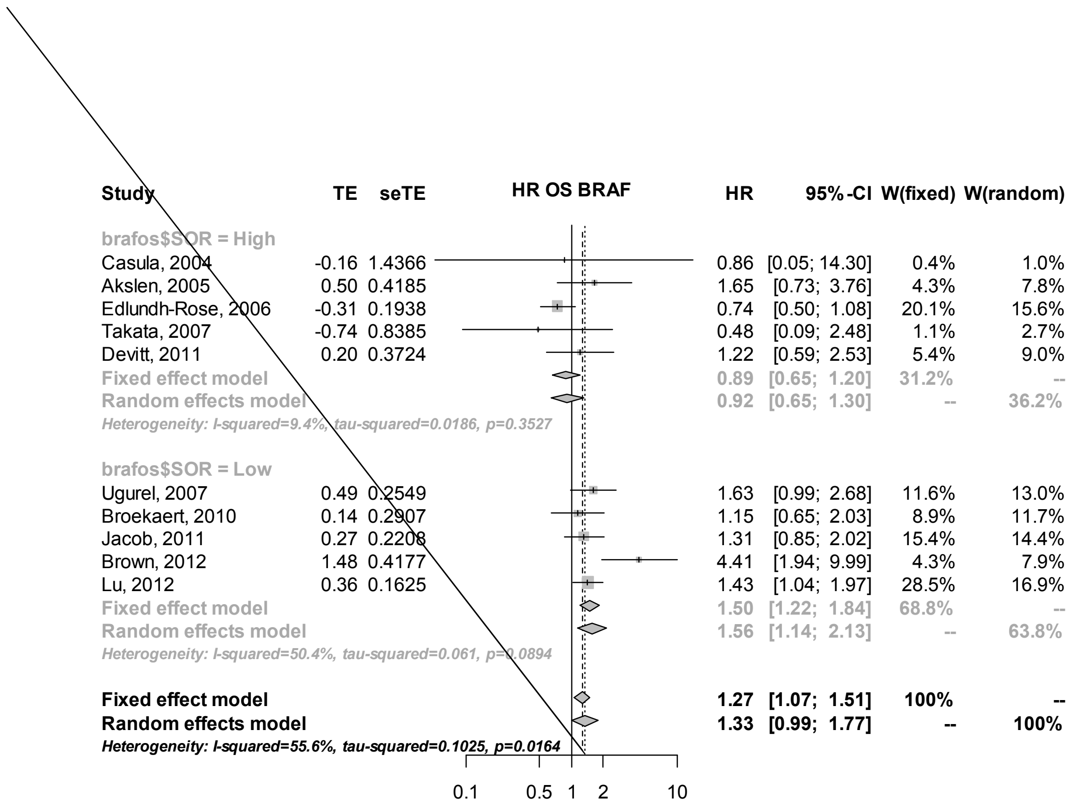

We have also divided the studies based on whether they enlisted only patients with metastatic disease or they included patients with all stages of melanoma. The later is a more homogenous group giving statistically significant results for both fixed and random-effects meta-analysis (HR=1.49 for the random effects model).

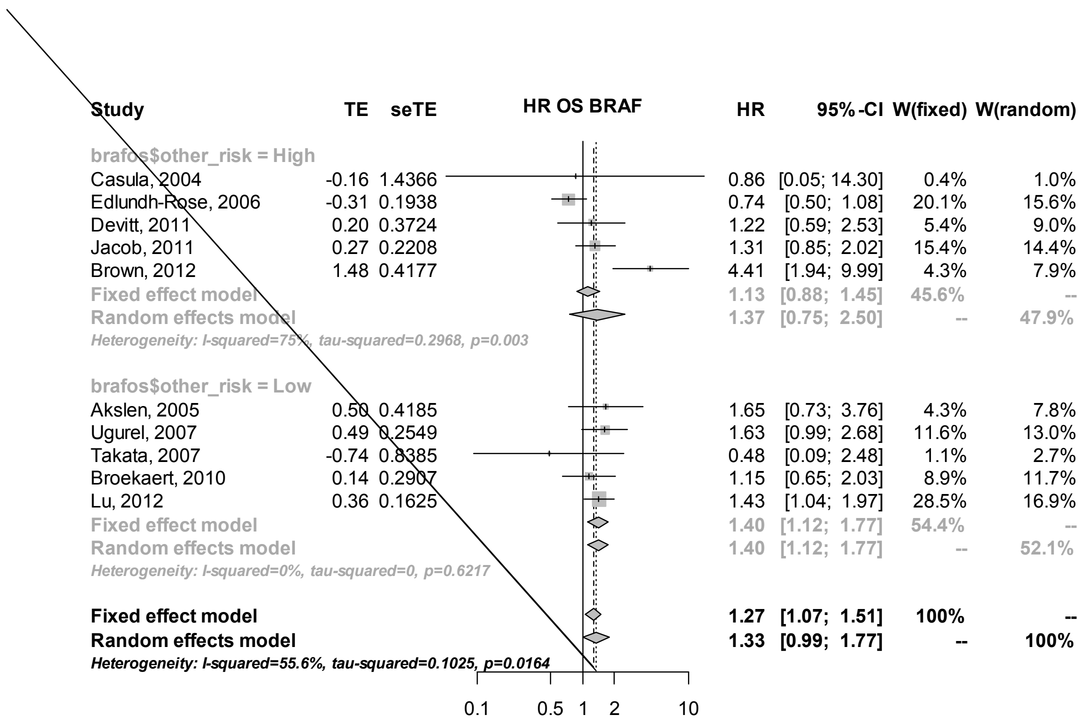

Study TE seTE HR OS BRAF HR 95%-CI W(fixed) W(random)
brafos$only_met_patients = 0
Casula, 2004 -0.16 1.4366 0.86 [0.05; 14.30] 0.4% 1.0%
Akslen, 2005 0.50 0.4185 1.65 [0.73; 3.76] 4.3% 7.8%
Takata, 2007 -0.74 0.8385 0.48 [0.09; 2.48] 1.1% 2.7%
Broekaert, 2010 0.14 0.2907 1.15 [0.65; 2.03] 8.9% 11.7%
Devitt, 2011 0.20 0.3724 1.22 [0.59; 2.53] 5.4% 9.0%
Brown, 2012 1.48 0.4177 4.41 [1.94; 9.99] 4.3% 7.9%
Lu, 2012 0.36 0.1625 1.43 [1.04; 1.97] 28.5% 16.9%
Fixed effect model 1.47 [1.16; 1.85] 52.9% --
Random effects model 1.49 [1.04; 2.15] -- 57.0%
Heterogeneity: I-squared=39.5%, tau-squared=0.0849, p=0.1281
brafos$only_met_patients = 1
Edlundh-Rose, 2006 -0.31 0.1938 0.74 [0.50; 1.08] 20.1% 15.6%
Ugurel, 2007 0.49 0.2549 1.63 [0.99; 2.68] 11.6% 13.0%
Jacob, 2011 0.27 0.2208 1.31 [0.85; 2.02] 15.4% 14.4%
Fixed effect model 1.08 [0.84; 1.38] 47.1% --
Random effects model 1.14 [0.71; 1.84] -- 43.0%
Heterogeneity: I-squared=72.4%, tau-squared=0.1289, p=0.0267
Fixed effect model 1.27 [1.07; 1.51] 100% --
Random effects model 1.33 [0.99; 1.77] -- 100%
Heterogeneity: I-squared=55.6%, tau-squared=0.1025, p=0.0164
0.1 0.5 1 2 10

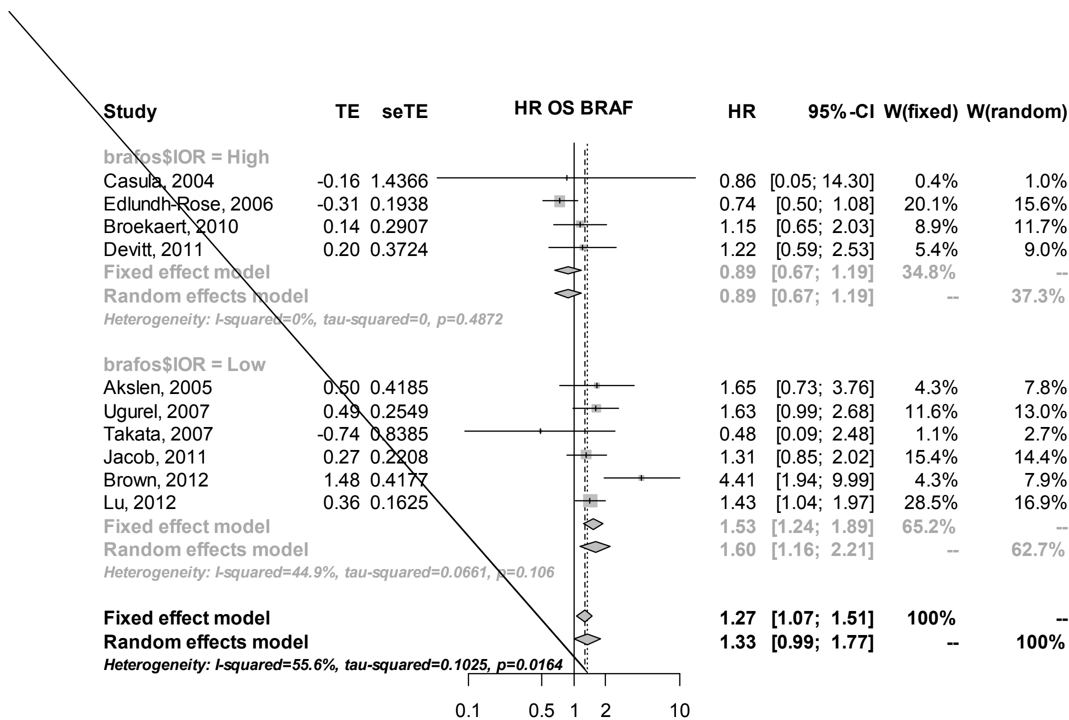
Subgrouping by whether the researchers analysed only exon 15 BRAF mutations or more does not give any statistical results under the random effects model.




We see below that studies with low selective outcome risk (SOR), incomplete outcome risk (IOR) and other risks tend to give a statistically significant summary effect under the random effects model with BRAF being negative to survival compared to wild-type tumours.


Study TE seTE HR OS BRAF HR 95%-CI W(fixed) W(random)
brafos$SOR = High
Casula, 2004 -0.16 1.4366 0.86 [0.05; 14.30] 0.4% 1.0%
Akslen, 2005 0.50 0.4185 1.65 [0.73; 3.76] 4.3% 7.8%
Edlundh-Rose, 2006 -0.31 0.1938 0.74 [0.50; 1.08] 20.1% 15.6%
Takata, 2007 -0.74 0.8385 0.48 [0.09; 2.48] 1.1% 2.7%
Devitt, 2011 0.20 0.3724 1.22 [0.59; 2.53] 5.4% 9.0%
Fixed effect model 0.89 [0.65; 1.20] 31.2% --
Random effects model 0.92 [0.65; 1.30] -- 36.2%
Heterogeneity: I-squared=9.4%, tau-squared=0.0186, p=0.3527
brafos$SOR = Low
Ugurel, 2007 0.49 0.2549 1.63 [0.99; 2.68] 11.6% 13.0%
Broekaert, 2010 0.14 0.2907 1.15 [0.65; 2.03] 8.9% 11.7%
Jacob, 2011 0.27 0.2208 1.31 [0.85; 2.02] 15.4% 14.4%
Brown, 2012 1.48 0.4177 4.41 [1.94; 9.99] 4.3% 7.9%
Lu, 2012 0.36 0.1625 1.43 [1.04; 1.97] 28.5% 16.9%
Fixed effect model 1.50 [1.22; 1.84] 68.8% --
Random effects model 1.56 [1.14; 2.13] -- 63.8%
Heterogeneity: I-squared=50.4%, tau-squared=0.061, p=0.0894
Fixed effect model 1.27 [1.07; 1.51] 100% --
Random effects model 1.33 [0.99; 1.77] -- 100%
Heterogeneity: I-squared=55.6%, tau-squared=0.1025, p=0.0164
0.1 0.5 1 2 10


Study TE seTE HR OS BRAF HR 95%-CI W(fixed) W(random)
brafos$other_risk = High
Casula, 2004 -0.16 1.4366 0.86 [0.05; 14.30] 0.4% 1.0%
Edlundh-Rose, 2006 -0.31 0.1938 0.74 [0.50; 1.08] 20.1% 15.6%
Devitt, 2011 0.20 0.3724 1.22 [0.59; 2.53] 5.4% 9.0%
Jacob, 2011 0.27 0.2208 1.31 [0.85; 2.02] 15.4% 14.4%
Brown, 2012 1.48 0.4177 4.41 [1.94; 9.99] 4.3% 7.9%
Fixed effect model 1.13 [0.88; 1.45] 45.6% --
Random effects model 1.37 [0.75; 2.50] -- 47.9%
Heterogeneity: I-squared=75%, tau-squared=0.2968, p=0.003
brafos$other_risk = Low
Akslen, 2005 0.50 0.4185 1.65 [0.73; 3.76] 4.3% 7.8%
Ugurel, 2007 0.49 0.2549 1.63 [0.99; 2.68] 11.6% 13.0%
Takata, 2007 -0.74 0.8385 0.48 [0.09; 2.48] 1.1% 2.7%
Broekaert, 2010 0.14 0.2907 1.15 [0.65; 2.03] 8.9% 11.7%
Lu, 2012 0.36 0.1625 1.43 [1.04; 1.97] 28.5% 16.9%
Fixed effect model 1.40 [1.12; 1.77] 54.4% --
Random effects model 1.40 [1.12; 1.77] -- 52.1%
Heterogeneity: I-squared=0%, tau-squared=0, p=0.6217
Fixed effect model 1.27 [1.07; 1.51] 100% --
Random effects model 1.33 [0.99; 1.77] -- 100%
Heterogeneity: I-squared=55.6%, tau-squared=0.1025, p=0.0164
0.1 0.5 1 2 10




By doing a meta-regression with the time from primary or metastatic tumour biopsy as a categorical value we find there is difference between the two groups but not statistically significant results for either one of them. First, we fixed a mixed-effects model, where the pooled summary effect estimate is calculated under the same $\tau^2$ but we pool a different summary effect for the different subgroups. The p-value for the "Test of Moderators" shows that we do not have a statistically significant variable and the significant p-value for the residual heterogeneity shows that our data are not explained by this model.

```
Mixed-Effects Model (k = 10; tau^2 estimator: DL)

  logLik  Deviance       AIC       BIC
 -7.6495   15.2989   21.2989   22.2067

tau^2 (estimate of residual amount of heterogeneity): 0.1283
tau (sqrt of the estimate of residual heterogeneity): 0.3582
```

```
Test for Residual Heterogeneity:
QE(df = 8) = 19.5053, p-val = 0.0124

Test of Moderators (coefficient(s) 1,2):
QM(df = 2) = 3.4194, p-val = 0.1809

Model Results:

                              estimate      se    zval    pval    ci.lb   ci.ub
factor(time_from)metastatic    0.3732  0.3040  1.2278  0.2195  -0.2225  0.9690
factor(time_from)primary       0.2559  0.1851  1.3827  0.1668  -0.1068  0.6186

factor(time_from)metastatic
factor(time_from)primary
```

The same is true for the random-effects model that follows:

```
Mixed-Effects Model (k = 10; tau^2 estimator: DL)

tau^2 (estimate of residual amount of heterogeneity): 0.1283
tau (sqrt of the estimate of residual heterogeneity): 0.3582

Test for Residual Heterogeneity:
QE(df = 8) = 19.5053, p-val = 0.0124

Test of Moderators (coefficient(s) 2):
QM(df = 1) = 0.1087, p-val = 0.7416

Model Results:

                        estimate      se     zval    pval    ci.lb   ci.ub
intrcpt                   0.3732  0.3040   1.2278  0.2195  -0.2225  0.9690
brafos$time_fromprimary  -0.1173  0.3559  -0.3297  0.7416  -0.8149  0.5802

---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

There is no statistical difference found for the variables of continent (Europe, North America, Oceania, Asia, North America and Europe), design (prospective or retrospective), fixation type (frozen, formalin or mixed), specimen type (primary

cutaneous, metastatic, mixed), analysing more exons than exon 15, whether the data came from Kaplan-Meier or Cox P-H, or whether the studies included only metastatic patients or not. However, for the last variable, there are statistically significant results for the group that used patients with all stages of melanoma.

## CONTINENT

```
Mixed-Effects Model (k = 10; tau^2 estimator: DL)

tau^2 (estimate of residual amount of heterogeneity): 0.3887
tau (sqrt of the estimate of residual heterogeneity): 0.6234

Test for Residual Heterogeneity:
QE(df = 5) = 19.7438, p-val = 0.0014

Test of Moderators (coefficient(s) 2,3,4,5):
QM(df = 4) = 0.4591, p-val = 0.9774

Model Results:

                            estimate      se    zval    pval    ci.lb   ci.ub
intrcpt                       0.0579  0.5484  0.1055  0.9159  -1.0169  1.1327
brafos$ContinentEurope        0.3907  0.6467  0.6041  0.5458  -0.8769  1.6583
brafos$ContinentNorthA        0.2121  0.8592  0.2469  0.8050  -1.4718  1.8961
brafos$ContinentNorthA_Eu     0.0793  0.8797  0.0901  0.9282  -1.6449  1.8034
brafos$ContinentOceania       0.1410  0.9100  0.1549  0.8769  -1.6426  1.9245

---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

## DESIGN

```
Mixed-Effects Model (k = 10; tau^2 estimator: DL)

tau^2 (estimate of residual amount of heterogeneity): 0.1223
```

```
tau (sqrt of the estimate of residual heterogeneity): 0.3497

Test for Residual Heterogeneity:
QE(df = 8) = 19.5978, p-val = 0.0120

Test of Moderators (coefficient(s) 2):
QM(df = 1) = 0.0738, p-val = 0.7859

Model Results:

                            estimate      se     zval    pval    ci.lb   ci.ub
intrcpt                       0.3660  0.3302   1.1084  0.2677  -0.2811  1.0131
brafos$designRetrospective   -0.1017  0.3744  -0.2716  0.7859  -0.8356  0.6322

intrcpt
brafos$designRetrospective

---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

## FIXATION

```
Mixed-Effects Model (k = 8; tau^2 estimator: DL)

tau^2 (estimate of residual amount of heterogeneity): 0.1050
tau (sqrt of the estimate of residual heterogeneity): 0.3240

Test for Residual Heterogeneity:
QE(df = 5) = 9.7783, p-val = 0.0818

Test of Moderators (coefficient(s) 2,3):
QM(df = 2) = 3.0990, p-val = 0.2124

Model Results:

                         estimate      se     zval    pval    ci.lb   ci.ub
intrcpt                   -0.3060  0.3776  -0.8105  0.4177  -1.0460  0.4340
brafos$fixationformalin    0.7186  0.4266   1.6846  0.0921  -0.1174  1.5546  .
brafos$fixationfrozen      0.7919  0.5590   1.4166  0.1566  -0.3038  1.8876
```

```
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

## SPECIMEN TYPE

```
Mixed-Effects Model (k = 10; tau^2 estimator: DL)

tau^2 (estimate of residual amount of heterogeneity): 0.1637
tau (sqrt of the estimate of residual heterogeneity): 0.4046

Test for Residual Heterogeneity:
QE(df = 7) = 17.7961, p-val = 0.0129

Test of Moderators (coefficient(s) 2,3):
QM(df = 2) = 0.1531, p-val = 0.9263

Model Results:

                             estimate      se     zval    pval    ci.lb   ci.ub
intrcpt                        0.3740  0.3319   1.1270  0.2598  -0.2764  1.0244
brafos$Specimen_Typepri_cut   -0.1501  0.4123  -0.3640  0.7158  -0.9582  0.6580
brafos$Specimen_Typepri_met   -0.0402  0.4791  -0.0840  0.9331  -0.9793  0.8988

intrcpt
brafos$Specimen_Typepri_cut
brafos$Specimen_Typepri_met

---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

## EXONS BRAF

```
Mixed-Effects Model (k = 10; tau^2 estimator: DL)

tau^2 (estimate of residual amount of heterogeneity): 0.1216
```

```
tau (sqrt of the estimate of residual heterogeneity): 0.3488

Test for Residual Heterogeneity:
QE(df = 8) = 18.9715, p-val = 0.0150

Test of Moderators (coefficient(s) 2):
QM(df = 1) = 0.5461, p-val = 0.4599

Model Results:

                        estimate      se     zval    pval    ci.lb   ci.ub
intrcpt                   0.3919  0.2107   1.8600  0.0629  -0.0211  0.8049  .
brafos$braf_exons_2more  -0.2307  0.3122  -0.7390  0.4599  -0.8425  0.3811

---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

## DATA FROM

```
Mixed-Effects Model (k = 10; tau^2 estimator: DL)

tau^2 (estimate of residual amount of heterogeneity): 0.1437
tau (sqrt of the estimate of residual heterogeneity): 0.3791

Test for Residual Heterogeneity:
QE(df = 7) = 18.1292, p-val = 0.0114

Test of Moderators (coefficient(s) 2,3):
QM(df = 2) = 0.0584, p-val = 0.9712

Model Results:

                    estimate      se    zval    pval    ci.lb   ci.ub
intrcpt               0.1989  0.5314  0.3742  0.7083  -0.8427  1.2404
brafos$data_fromIPD   0.1303  0.5892  0.2212  0.8249  -1.0245  1.2852
brafos$data_fromKM    0.0740  0.5807  0.1275  0.8986  -1.0642  1.2123
```

```
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

## METASTATIC PATIENTS ONLY OR NOT

```
Mixed-Effects Model (k = 10; tau^2 estimator: DL)

tau^2 (estimate of residual amount of heterogeneity): 0.1055
tau (sqrt of the estimate of residual heterogeneity): 0.3249

Test for Residual Heterogeneity:
QE(df = 8) = 17.1637, p-val = 0.0284

Test of Moderators (coefficient(s) 2):
QM(df = 1) = 0.8365, p-val = 0.3604

Model Results:

                            estimate      se     zval    pval    ci.lb   ci.ub
intrcpt                       0.4025  0.1970   2.0431  0.0410   0.0164  0.7886
brafos$only_met_patientsyes  -0.2752  0.3009  -0.9146  0.3604  -0.8649  0.3145

intrcpt                     *
brafos$only_met_patientsyes

---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Whether the studies exhibit high or low risk of incomplete outcome bias, selective outcome or bias or other bias seems to be significant, dividing the studies between high and low quality. We have already noticed from the subgroup analysis that high quality studies give statistically significant results. Results are shown below.

## INCOMPLETE OUTCOME RISK

```
Mixed-Effects Model (k = 10; tau^2 estimator: DL)

tau^2 (estimate of residual amount of heterogeneity): 0.0398
tau (sqrt of the estimate of residual heterogeneity): 0.1994

Test for Residual Heterogeneity:
QE(df = 8) = 11.5133, p-val = 0.1743

Test of Moderators (coefficient(s) 2):
QM(df = 1) = 4.7376, p-val = 0.0295

Model Results:

               estimate      se     zval    pval    ci.lb   ci.ub
intrcpt         -0.0670  0.1922  -0.3486  0.7274  -0.4438  0.3098
brafos$IORLow    0.5260  0.2417   2.1766  0.0295   0.0524  0.9997  *

---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

## SELECTIVE OUTCOME RISK

```
Mixed-Effects Model (k = 10; tau^2 estimator: DL)

tau^2 (estimate of residual amount of heterogeneity): 0.0504
tau (sqrt of the estimate of residual heterogeneity): 0.2245

Test for Residual Heterogeneity:
QE(df = 8) = 12.4760, p-val = 0.1312

Test of Moderators (coefficient(s) 2):
QM(df = 1) = 3.6803, p-val = 0.0551

Model Results:

               estimate      se     zval    pval    ci.lb   ci.ub
intrcpt         -0.0544  0.2090  -0.2602  0.7947  -0.4640  0.3553
brafos$SORLow    0.4951  0.2581   1.9184  0.0551  -0.0107  1.0009  .
```

```
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

## OTHER RISK

```
Mixed-Effects Model (k = 10; tau^2 estimator: DL)

tau^2 (estimate of residual amount of heterogeneity): 0.1222
tau (sqrt of the estimate of residual heterogeneity): 0.3496

Test for Residual Heterogeneity:
QE(df = 8) = 18.6528, p-val = 0.0168

Test of Moderators (coefficient(s) 2):
QM(df = 1) = 0.0098, p-val = 0.9210

Model Results:

                      estimate      se    zval    pval    ci.lb   ci.ub
intrcpt                 0.2708  0.2249  1.2041  0.2285  -0.1700  0.7116
brafos$other_riskLow    0.0309  0.3116  0.0991  0.9210  -0.5799  0.6417

---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Interestingly enough, when we combine the variables of metastatic only patients and specimen type we get highly significant results and explain most of our data. What happens here is that we create 6 categories. We have 3 categories depending on whether the studies examined primary melanomas, metastatic or both and each of these categories is divided in another 2 depending on there were only metastatic patients included or not. Obviously the studies that examined metastatic melanomas will have only metastatic patients and that is one category of its own. That is why we do not see the variable of specimen type of metastatic on its own in the second mixed effects meta-analysis. It is the same

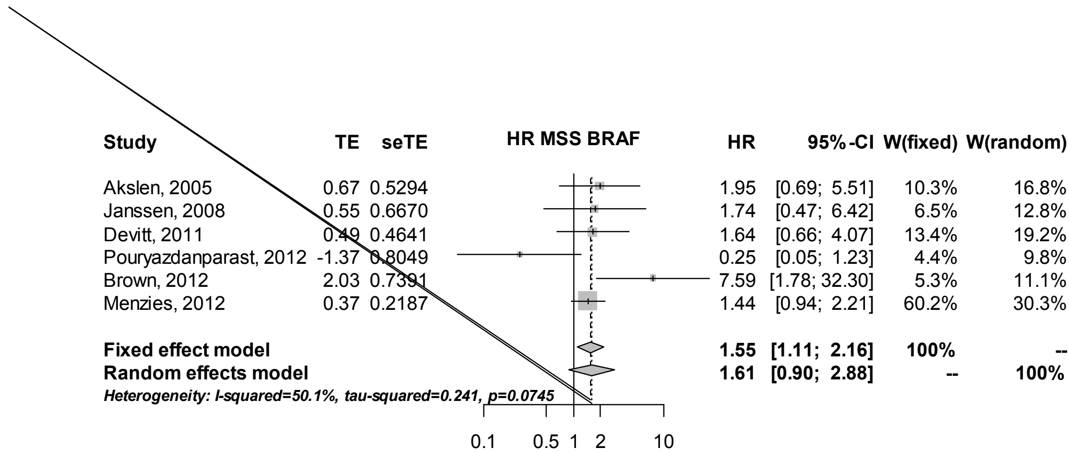
as the use of only metastatic patients. However, we have only 10 studies and only 5 categories, so the numbers are very few for this analysis to be robust. All it shows is that most of the heterogeneity between the studies is as to whether they used only primary tissue for analysis (and therefore possibly include all stages of melanoma) or only metastatic tissue (and therefore more likely to measure survival from the time of metastasis) or both. We show from above that the group that used both primary and metastatic tissue is the most heterogenous and we now understand better the reasons for that.

## METASTATIC ONLY PATIENTS AND SPECIMEN TYPE

```
Mixed-Effects Model (k = 10; tau^2 estimator: DL)

tau^2 (estimate of residual amount of heterogeneity): 0
tau (sqrt of the estimate of residual heterogeneity): 0

Test for Residual Heterogeneity:
QE(df = 6) = 3.8866, p-val = 0.6920

Test of Moderators (coefficient(s) 2,3,4):
QM(df = 3) = 16.3644, p-val = 0.0010

Model Results:

                             estimate      se     zval    pval    ci.lb
intrcpt                        2.0238  0.4757   4.2547  <.0001   1.0915
brafos$only_met_patientsyes   -1.6613  0.4454  -3.7296  0.0002  -2.5343
brafos$Specimen_Typepri_cut   -1.7349  0.4918  -3.5275  0.0004  -2.6988
brafos$Specimen_Typepri_met   -0.6686  0.2557  -2.6142  0.0089  -1.1698
                               ci.ub
intrcpt                       2.9561  ***
brafos$only_met_patientsyes  -0.7882  ***
brafos$Specimen_Typepri_cut  -0.7709  ***
brafos$Specimen_Typepri_met  -0.1673   **

---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
Mixed-Effects Model (k = 10; tau^2 estimator: DL)

tau^2 (estimate of residual amount of heterogeneity): 0
tau (sqrt of the estimate of residual heterogeneity): 0

Test for Residual Heterogeneity:
QE(df = 6) = 3.8866, p-val = 0.6920

Test of Moderators (coefficient(s) 1,2,3,4):
QM(df = 4) = 23.9791, p-val < .0001

Model Results:

                             estimate      se     zval    pval    ci.lb
brafos$only_met_patientsno     2.0238  0.4757   4.2547  <.0001   1.0915
brafos$only_met_patientsyes    0.3626  0.1669   2.1724  0.0298   0.0355
brafos$Specimen_Typepri_cut   -1.7349  0.4918  -3.5275  0.0004  -2.6988
brafos$Specimen_Typepri_met   -0.6686  0.2557  -2.6142  0.0089  -1.1698
                               ci.ub
brafos$only_met_patientsno    2.9561  ***
brafos$only_met_patientsyes   0.6897    *
brafos$Specimen_Typepri_cut  -0.7709  ***
brafos$Specimen_Typepri_met  -0.1673   **

---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

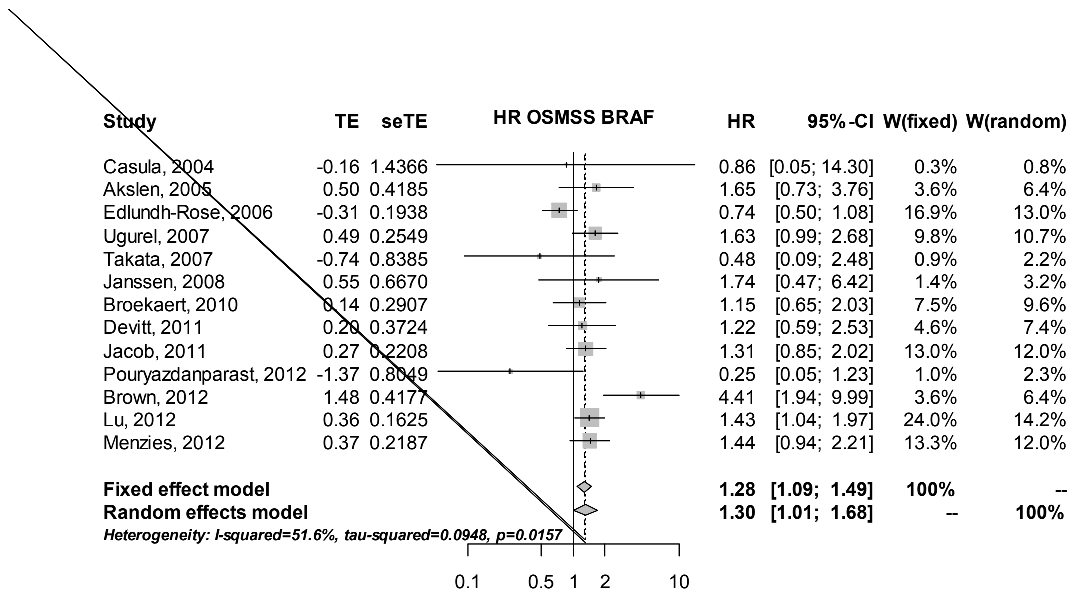
Analysis of the melanoma specific survival (MSS) does not give statistically significant results under the random-effects model.




However as we said above we feel that it is right to combine studies that report OS with studies that report MSS to increase our sample size, as we are not sure that all researchers indeed mean OS or MSS, apart from 3 out of 22 who specifically report both. We create one category where we use the OS if it is reported and the MSS if there is no available OS. By doing that we increase our sample size and we possible underestimate survival (higher HRs) due to more studies with OS that are included. However, we are not sure that the MSS is MSS for the studies that report it or just OS. Since it is more difficult to record MSS it would be more appropriate to record both OS and MSS, as 3 studies have done, than MSS alone. In any case, we get statistically significant results under the random-effects model with HR=1.30, p-value=0.0448). The analysis and the forest plot follow below:

```
Review:     HR_osmss for BRAF
                           TE seTE
Casula, 2004            -0.16 1.44
Akslen, 2005             0.50 0.42
Edlundh-Rose, 2006      -0.31 0.19
Ugurel, 2007             0.49 0.25
```

```
Takata, 2007          -0.74 0.84
Janssen, 2008          0.55 0.67
Broekaert, 2010        0.14 0.29
Devitt, 2011           0.20 0.37
Jacob, 2011            0.27 0.22
Pouryazdanparast, 2012 -1.37 0.80
Brown, 2012            1.48 0.42
Lu, 2012               0.36 0.16
Menzies, 2012          0.37 0.22


                         HR          95%-CI %W(fixed) %W(random)
Casula, 2004           0.86  [0.05; 14.30]      0.31       0.80
Akslen, 2005           1.65  [0.73;  3.76]      3.63       6.36
Edlundh-Rose, 2006     0.74  [0.50;  1.08]     16.91      12.97
Ugurel, 2007           1.63  [0.99;  2.68]      9.77      10.75
Takata, 2007           0.48  [0.09;  2.48]      0.90       2.15
Janssen, 2008          1.74  [0.47;  6.42]      1.43       3.18
Broekaert, 2010        1.15  [0.65;  2.03]      7.52       9.58
Devitt, 2011           1.22  [0.59;  2.53]      4.58       7.35
Jacob, 2011            1.31  [0.85;  2.02]     13.02      11.96
Pouryazdanparast, 2012 0.25  [0.05;  1.23]      0.98       2.31
Brown, 2012            4.41  [1.94;  9.99]      3.64       6.38
Lu, 2012               1.43  [1.04;  1.97]     24.05      14.17
Menzies, 2012          1.44  [0.94;  2.21]     13.27      12.04

Number of studies combined: k=13

                       HR        95%-CI    z  p.value
Fixed effect model   1.28  [1.09; 1.49] 3.07   0.0021
Random effects model 1.30  [1.01; 1.68] 2.01   0.0448

Quantifying heterogeneity:
tau^2 = 0.0948; H = 1.44 [1.05; 1.97]; I^2 = 51.6% [8.9%; 74.3%]

Test of heterogeneity:
     Q d.f.  p.value
 24.82   12   0.0157

Details on meta-analytical method:
- Inverse variance method
- DerSimonian-Laird estimator for tau^2
```




## Egger's test:

```
Review:     HR_osmss for BRAF

        Linear regression test of funnel plot asymmetry

data:  brafosmss.meta
t = -0.1699, df = 11, p-value = 0.8682
alternative hypothesis: asymmetry in funnel plot
sample estimates:
      bias    se.bias      slope
-0.1495800  0.8804901  0.2827971
```

## Peters test:

```
Regression Test for Funnel Plot Asymmetry

model:     mixed-effects meta-regression model
predictor: inverse of the total sample size

z = -3.0523, p = 0.0023
```

The Egger's test does not show heterogeneity but we know that is not true and Peters test is highly suggestive of heterogeneity with p=0.0023.

An influential analysis show that the studies by Edlundh-Rose et al. (2006) and Brown et al. (2012) are the ones increasing heterogeneity significantly. The study by Edlundh-Rose et al. includes only patients who have progressed to metastatic disease and both the above studies analysed BRAF from specimens from primary and metastatic tissue.

```
Influential analysis (Random effects model)

                                     HR           95%-CI  p.value    tau^2
Omitting Casula, 2004            1.3053  [1.0031; 1.6986]   0.0474   0.102
Omitting Akslen, 2005            1.2792  [0.9738; 1.6804]   0.0769   0.1041
Omitting Edlundh-Rose, 2006      1.4265  [1.1417; 1.7822]   0.0018   0.0374
Omitting Ugurel, 2007            1.2664  [0.9553; 1.6788]   0.1006   0.1077
Omitting Takata, 2007            1.3294  [1.0268; 1.7212]   0.0307   0.0931
Omitting Janssen, 2008           1.2881  [0.9862; 1.6823]   0.0632   0.1025
Omitting Broekaert, 2010         1.3181  [0.9931; 1.7495]   0.0559   0.1115
Omitting Devitt, 2011            1.3075  [0.9906; 1.7257]   0.0584   0.1084
Omitting Jacob, 2011             1.2990  [0.9689; 1.7415]   0.0803   0.1206
Omitting Pouryazdanparast, 2012  1.3456  [1.0566; 1.7137]   0.0161   0.0729
Omitting Brown, 2012             1.2105  [0.9810; 1.4937]   0.0749   0.0365
Omitting Lu, 2012                1.2807  [0.9472; 1.7318]   0.108    0.1286
Omitting Menzies, 2012           1.2826  [0.9584; 1.7165]   0.0941   0.118

Pooled estimate                  1.3008  [1.0062; 1.6817]   0.0448   0.0948
                                     I^2
Omitting Casula, 2004              55.5%
Omitting Akslen, 2005              55.0%
Omitting Edlundh-Rose, 2006        27.1%
Omitting Ugurel, 2007              53.8%
Omitting Takata, 2007              53.1%
Omitting Janssen, 2008             55.3%
Omitting Broekaert, 2010           55.4%
Omitting Devitt, 2011              55.6%
Omitting Jacob, 2011               55.7%
Omitting Pouryazdanparast, 2012    46.9%
Omitting Brown, 2012               29.9%
```

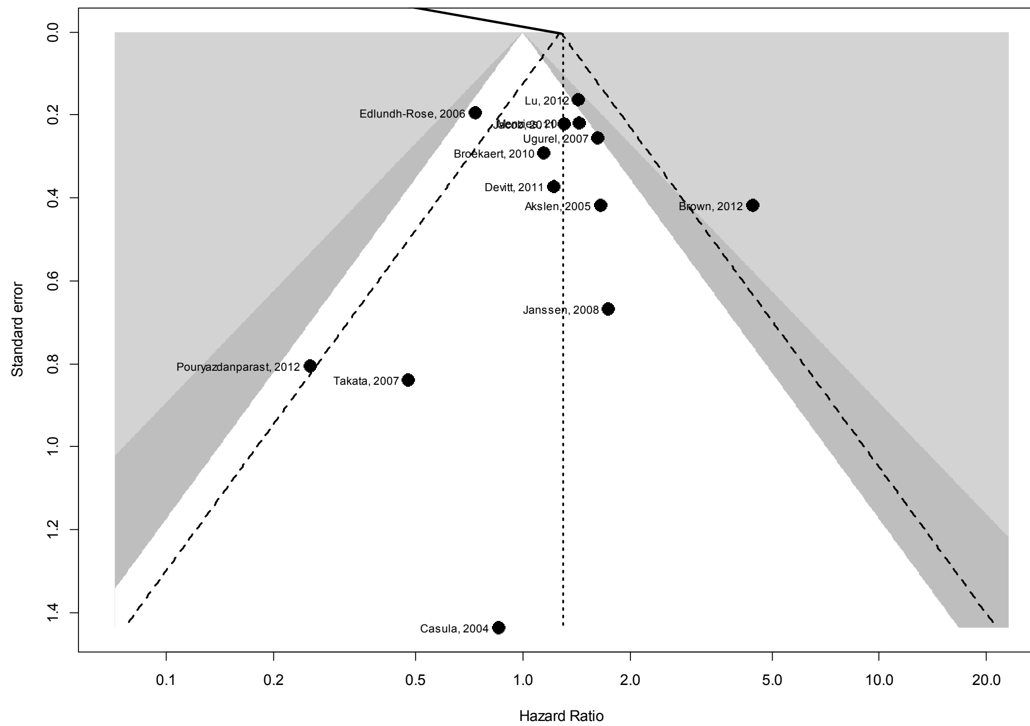
```
Omitting Lu, 2012                     54.5%
Omitting Menzies, 2012                55.0%

Pooled estimate                       51.6%

Details on meta-analytical method:
- Inverse variance method
- DerSimonian-Laird estimator for tau^2
```

By doing a cumulative meta-analysis we see that the latest studies by Lu et al. (2012) and Menzies et al. (2012) were significant for this meta-analysis to reach statistical significance.

```
Cumulative meta-analysis (Random effects model)

                                          HR            95%-CI  p.value
Adding Casula, 2004 (k=1)             0.8563  [0.0513; 14.3046]   0.914
Adding Akslen, 2005 (k=2)             1.5716  [0.7150;  3.4542]   0.2605
Adding Edlundh-Rose, 2006 (k=3)       0.9631  [0.5333;  1.7394]   0.9008
Adding Ugurel, 2007 (k=4)             1.1713  [0.6785;  2.0219]   0.5703
Adding Takata, 2007 (k=5)             1.0886  [0.6562;  1.8059]   0.7424
Adding Janssen, 2008 (k=6)            1.1381  [0.7224;  1.7928]   0.577
Adding Broekaert, 2010 (k=7)          1.1270  [0.7965;  1.5945]   0.4997
Adding Devitt, 2011 (k=8)             1.1262  [0.8403;  1.5094]   0.4263
Adding Jacob, 2011 (k=9)              1.1521  [0.9050;  1.4667]   0.2502
Adding Pouryazdanparast, 2012 (k=10)  1.1180  [0.8494;  1.4715]   0.4263
Adding Brown, 2012 (k=11)             1.2564  [0.8868;  1.7800]   0.199
Adding Lu, 2012 (k=12)                1.2826  [0.9584;  1.7165]   0.0941
Adding Menzies, 2012 (k=13)           1.3008  [1.0062;  1.6817]   0.0448

Pooled estimate                       1.3008  [1.0062;  1.6817]   0.0448
                                         tau^2     I^2
Adding Casula, 2004 (k=1)               0
Adding Akslen, 2005 (k=2)               0          0.0%
Adding Edlundh-Rose, 2006 (k=3)         0.1058   35.1%
Adding Ugurel, 2007 (k=4)               0.161    60.0%
Adding Takata, 2007 (k=5)               0.1451   52.0%
Adding Janssen, 2008 (k=6)              0.1198   44.1%
Adding Broekaert, 2010 (k=7)            0.0656   33.5%
Adding Devitt, 2011 (k=8)               0.0395   23.4%
```

```
Adding Jacob, 2011 (k=9)                  0.0236   17.9%
Adding Pouryazdanparast, 2012 (k=10)      0.0545   31.4%
Adding Brown, 2012 (k=11)                 0.1647   57.5%
Adding Lu, 2012 (k=12)                    0.118    55.0%
Adding Menzies, 2012 (k=13)               0.0948   51.6%

Pooled estimate                           0.0948   51.6%

Details on meta-analytical method:
- Inverse variance method
- DerSimonian-Laird estimator for tau^2
```

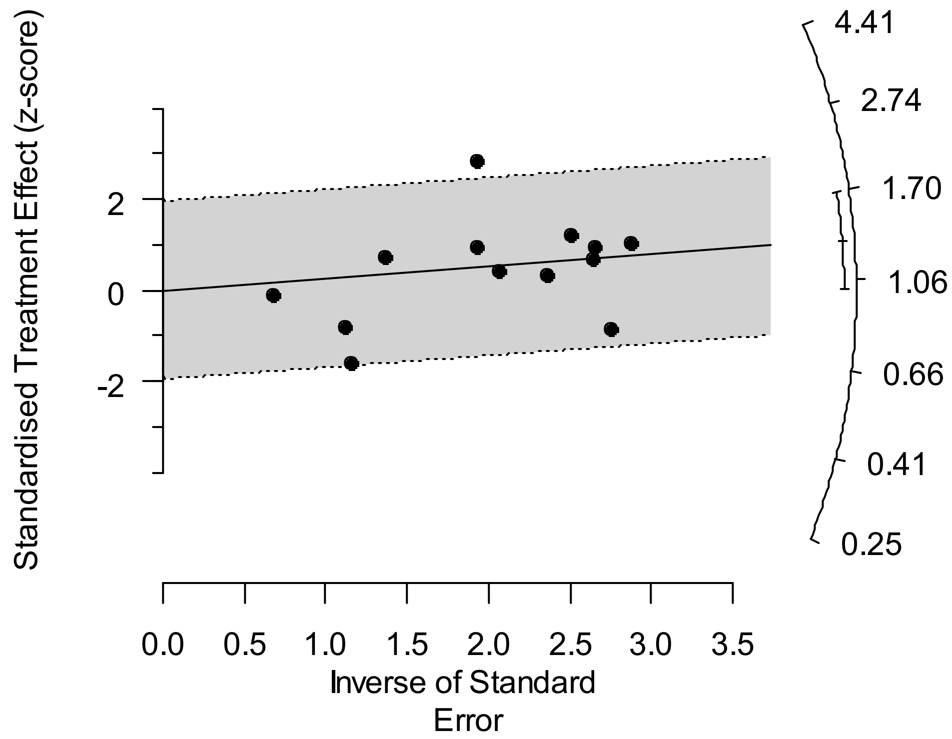
A contour enhanced funnel plot below shows that we may be missing some small studies with large positive or negative HRs and big standard error, but as we see by the trim and fill analysis further below, it seems that we have enough studies and no significant publication bias is identified.




```
                     HR            95%-CI %W(fixed) %W(random)
```

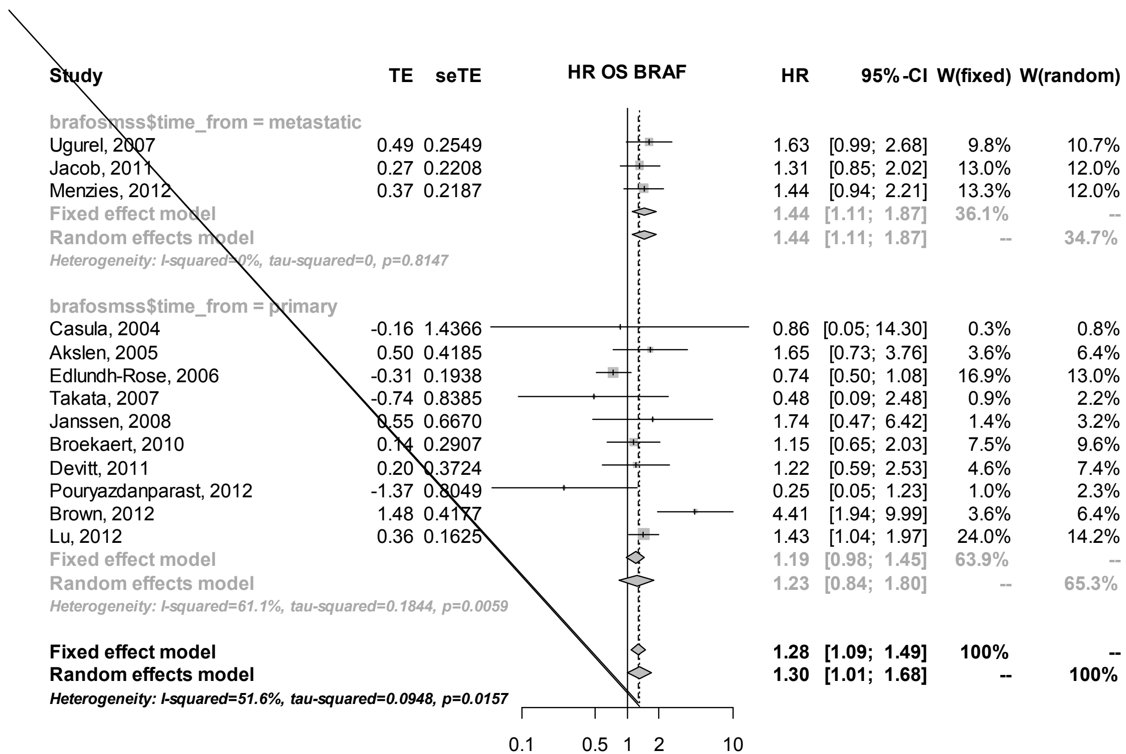
```
Broekaert, 2010        1.1470  [0.6489;  2.0276]      7.52       9.58
Devitt, 2011           1.2200  [0.5880;  2.5313]      4.58       7.35
Janssen, 2008          1.7376  [0.4702;  6.4221]      1.43       3.18
Edlundh-Rose, 2006     0.7364  [0.5037;  1.0766]     16.91      12.97
Ugurel, 2007           1.6256  [0.9864;  2.6791]      9.77      10.75
Pouryazdanparast, 2012 0.2532  [0.0523;  1.2263]      0.98       2.31
Jacob, 2011            1.3100  [0.8498;  2.0195]     13.02      11.96
Brown, 2012            4.4058  [1.9431;  9.9898]      3.64       6.38
Lu, 2012               1.4329  [1.0421;  1.9703]     24.05      14.17
Akslen, 2005           1.6547  [0.7286;  3.7578]      3.63       6.36
Menzies, 2012          1.4421  [0.9393;  2.2139]     13.27      12.04
Takata, 2007           0.4790  [0.0926;  2.4779]      0.90       2.15
Casula, 2004           0.8563  [0.0513; 14.3046]      0.31       0.80

Number of studies combined: k=13 (with 0 added studies)

                         HR            95%-CI      z  p.value
Fixed effect model   1.2775  [1.0928; 1.4935] 3.0736   0.0021
Random effects model 1.3008  [1.0062; 1.6817] 2.0068   0.0448

Quantifying heterogeneity:
tau^2 = 0.0948; H = 1.44 [1.05; 1.97]; I^2 = 51.6% [8.9%; 74.3%]

Test of heterogeneity:
     Q d.f.  p.value
 24.82   12   0.0157

Details on meta-analytical method:
- Inverse variance method
- DerSimonian-Laird estimator for tau^2
- Trim-and-fill method to adjust for funnel plot asymmetry
```

The following radial plot shows that we have heterogeneity, and the study by Brown et al. (2012) is the one that stands out of the rest.


Standardised Treatment Effect (z-score)
2
0
-2
4.41
2.74
1.70
1.06
0.66
0.41
0.25
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
Inverse of Standard
Error

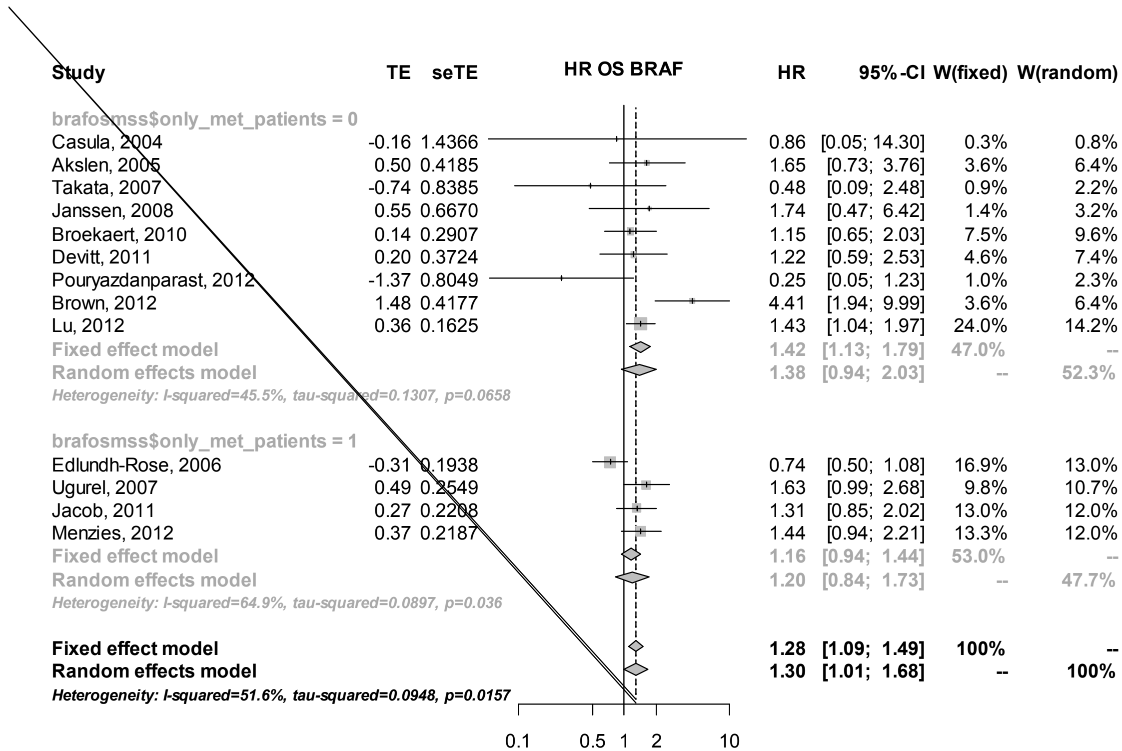
We repeated the subgroup analysis for all the variables that we examined in the OS analysis and we present the subgroup analysis for the variables of measuring survival from the time of diagnosis of primary or metastatic disease, for the studies that used only patients who had metastasis and for the studies grouped by specimen type: primary cutaneous, metastatic, mixed, ocular.

We notice that measuring time from the time of metastatic diagnosis, although it includes only 3 studies, it is a fairly homogenous group and gives a statistically significant result under the fixed effects model (HR=1.44, 1.11-1.87, p-value=0.0061). The measuring of time for the diagnosis of primary disease does not yet give a statistically significant result, although there is a trend towards HRs >1.

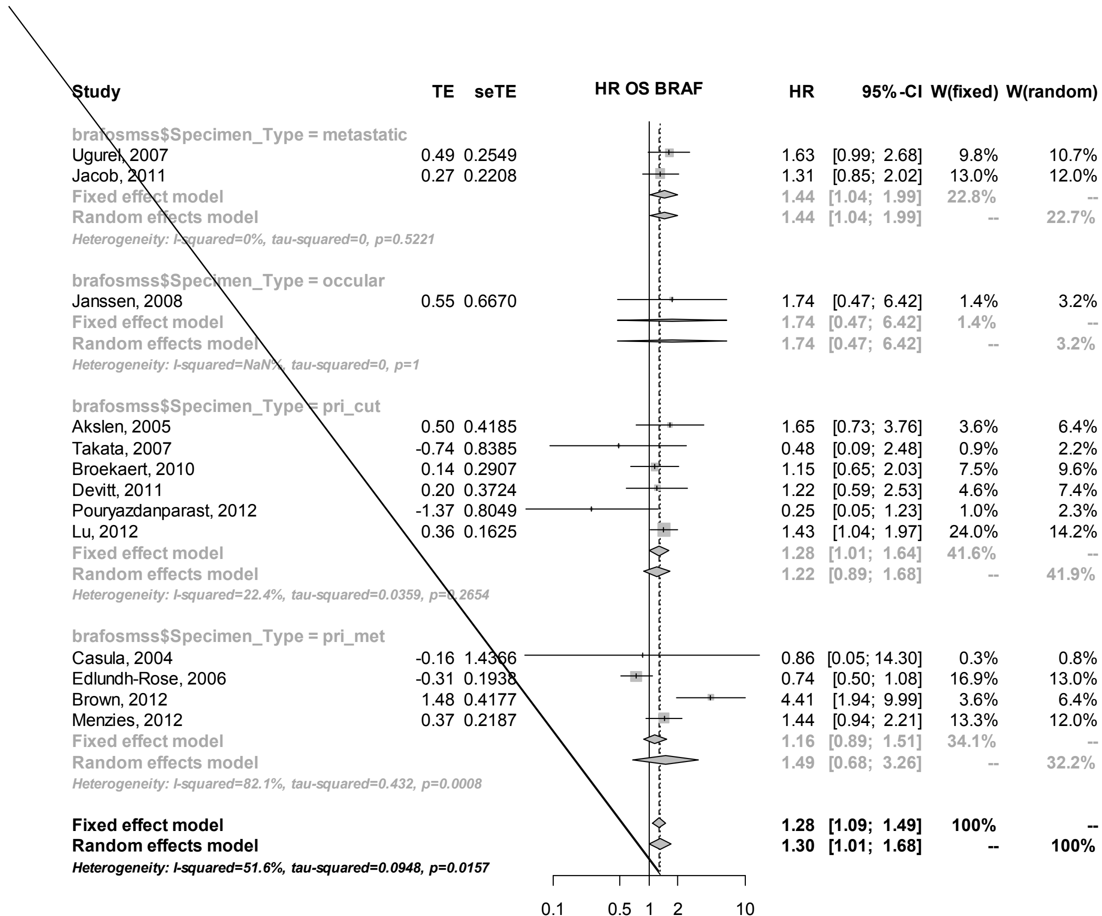


```
Review:     HR_osmss for BRAF
                TE seTE
Ugurel, 2007  0.49 0.25
Jacob, 2011   0.27 0.22
Menzies, 2012 0.37 0.22


                HR        95%-CI %W(fixed) %W(random)
Ugurel, 2007  1.63  [0.99; 2.68]      27.1       27.1
Jacob, 2011   1.31  [0.85; 2.02]      36.1       36.1
Menzies, 2012 1.44  [0.94; 2.21]      36.8       36.8

Number of studies combined: k=3

                       HR        95%-CI    z  p.value
Fixed effect model   1.44  [1.11; 1.87] 2.74   0.0061
Random effects model 1.44  [1.11; 1.87] 2.74   0.0061

Quantifying heterogeneity:
tau^2 < 0.0001; H = 1 [1; 1.4]; I^2 = 0% [0%; 49.2%]

Test of heterogeneity:
    Q d.f.  p.value
 0.41    2   0.8147

Details on meta-analytical method:
- Inverse variance method
- DerSimonian-Laird estimator for tau^2
```

Grouping the studies by essentially the stage of the patients they included, did not give any significant results.



We got significant results from the group that analysed only metastatic tissue, but that affects only two studies and we do not consider it strong enough for an honest suggestion. However, it supports the trend we are seeing that BRAF may affect the survival in only the metastatic patients.



## Disease Free Survival (DFS) of patients with BRAF and wild type (WT) mutations

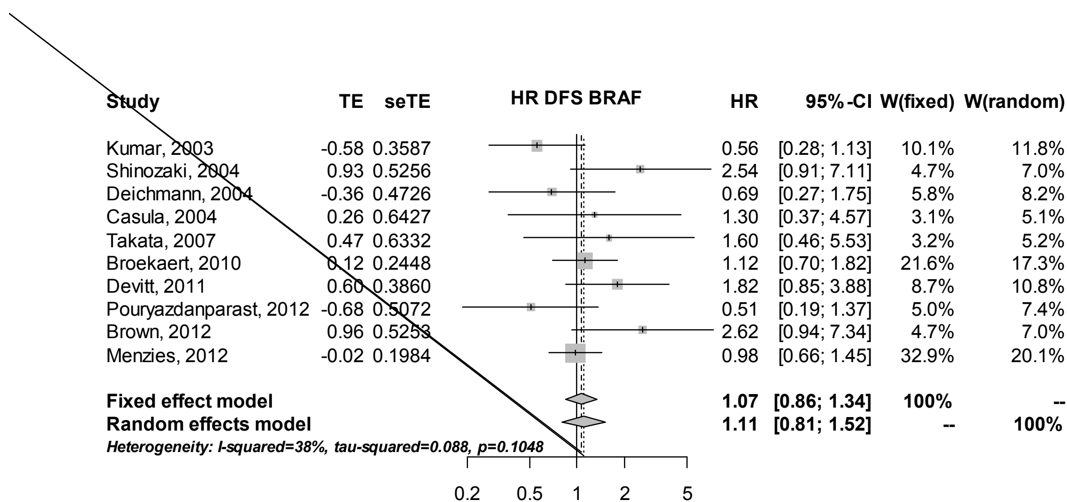
Below we present the analysis for the DFS of patients with BRAF mutated melanomas. No statistical significance was found. The tables 15-17 above present the included studies with their characteristics. Unlike to the overall and melanoma specific survival above, there is not so much heterogeneity in the DFS studies because they have to measure time from the diagnosis of the primary melanoma and they have to examine primary melanoma tissue for mutations.:

Review: HR_DFS for BRAF

| | TE | seTE |
|---|---|---|
| Kumar, 2003 | -0.58 | 0.36 |
| Shinozaki, 2004 | 0.93 | 0.53 |
| Deichmann, 2004 | -0.36 | 0.47 |
| Casula, 2004 | 0.26 | 0.64 |
| Takata, 2007 | 0.47 | 0.63 |
| Broekaert, 2010 | 0.12 | 0.24 |
| Devitt, 2011 | 0.60 | 0.39 |
| Pouryazdanparast, 2012 | -0.68 | 0.51 |
| Brown, 2012 | 0.96 | 0.53 |
| Menzies, 2012 | -0.02 | 0.20 |

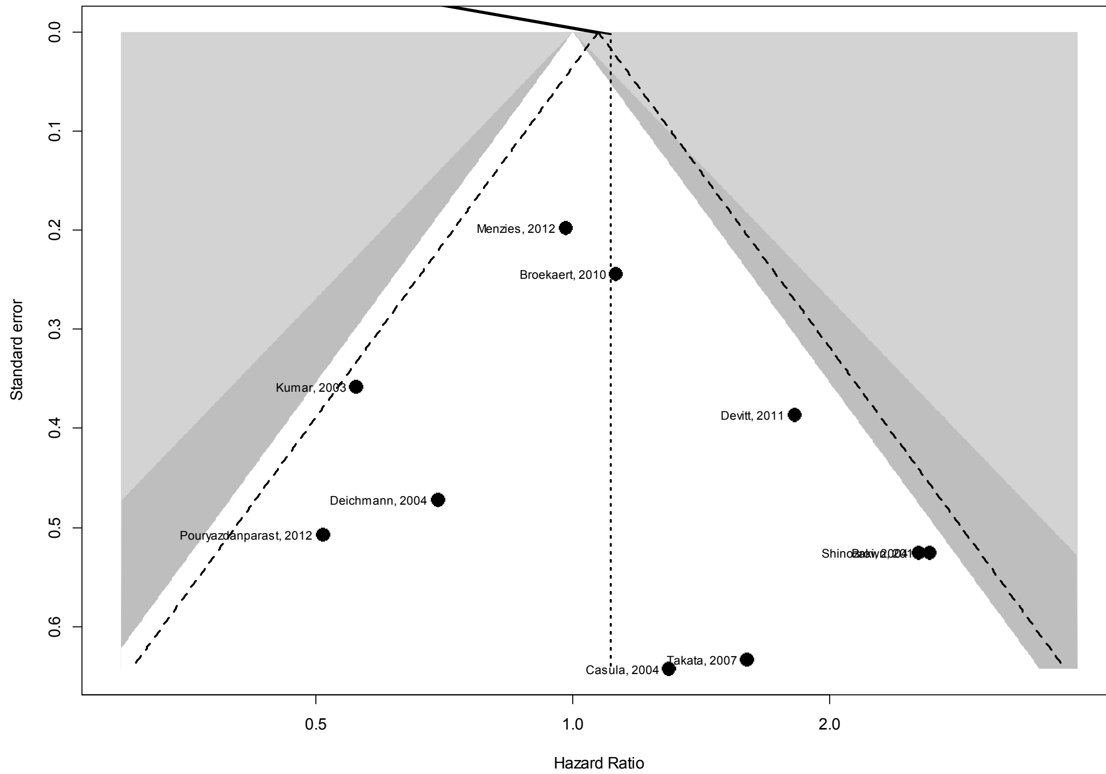
| | HR | 95%-CI | %W(fixed) | %W(random) |
|---|---|---|---|---|
| Kumar, 2003 | 0.56 | [0.28; 1.13] | 10.08 | 11.81 |
| Shinozaki, 2004 | 2.54 | [0.91; 7.11] | 4.69 | 7.02 |
| Deichmann, 2004 | 0.69 | [0.27; 1.75] | 5.81 | 8.22 |
| Casula, 2004 | 1.30 | [0.37; 4.57] | 3.14 | 5.11 |

```
Takata, 2007            1.60  [0.46; 5.53]      3.24       5.23
Broekaert, 2010         1.12  [0.70; 1.82]     21.65      17.29
Devitt, 2011            1.82  [0.85; 3.88]      8.70      10.79
Pouryazdanparast, 2012  0.51  [0.19; 1.37]      5.04       7.41
Brown, 2012             2.62  [0.94; 7.34]      4.70       7.03
Menzies, 2012           0.98  [0.66; 1.45]     32.95      20.08

Number of studies combined: k=10

                        HR        95%-CI     z  p.value
Fixed effect model    1.07  [0.86; 1.34] 0.60   0.5458
Random effects model  1.11  [0.81; 1.52] 0.64   0.5213

Quantifying heterogeneity:
tau^2 = 0.0880; H = 1.27 [1; 1.84]; I^2 = 38% [0%; 70.5%]

Test of heterogeneity:
     Q d.f.  p.value
 14.53    9   0.1048

Details on meta-analytical method:
- Inverse variance method
- DerSimonian-Laird estimator for tau^2
```



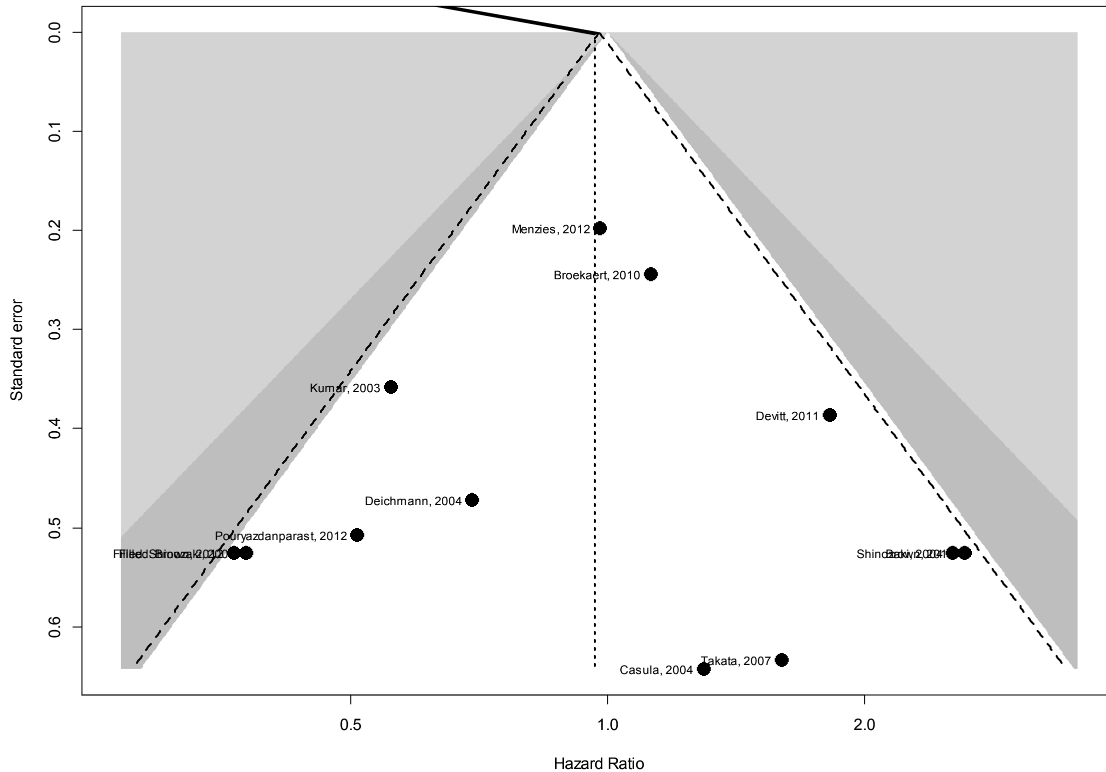
```
Review:     HR_DFS for
BRAF

        Linear regression test of funnel plot asymmetry

data:  brafdfs.meta
t = 0.7313, df = 8, p-value = 0.4854
alternative hypothesis: asymmetry in funnel plot
sample estimates:
      bias    se.bias      slope
 0.7678450  1.0499034 -0.1855061



Influential analysis (Random effects model)

                                      HR           95%-CI  p.value    tau^2
Omitting Kumar, 2003              1.1992  [0.8866; 1.6221]   0.2383   0.0522
Omitting Shinozaki, 2004          1.0376  [0.7658; 1.4059]   0.8117   0.0631
Omitting Deichmann, 2004          1.1589  [0.8291; 1.6199]   0.3881   0.0976
Omitting Casula, 2004             1.1031  [0.7878; 1.5447]   0.5679   0.1073
Omitting Takata, 2007             1.0885  [0.7802; 1.5186]   0.6177   0.102
Omitting Broekaert, 2010          1.1183  [0.7648; 1.6351]   0.5641   0.1386
Omitting Devitt, 2011             1.0417  [0.7520; 1.4429]   0.8059   0.0806
Omitting Pouryazdanparast, 2012   1.1720  [0.8568; 1.6031]   0.3206   0.073
Omitting Brown, 2012              1.0348  [0.7663; 1.3974]   0.8235   0.0595
Omitting Menzies, 2012            1.1527  [0.7818; 1.6995]   0.4733   0.1453

Pooled estimate                   1.1080  [0.8098; 1.5160]   0.5213   0.088
                                      I^2
Omitting Kumar, 2003                26.2%
Omitting Shinozaki, 2004            31.6%
Omitting Deichmann, 2004            41.3%
Omitting Casula, 2004               44.6%
Omitting Takata, 2007               43.3%
Omitting Broekaert, 2010            44.7%
Omitting Devitt, 2011               35.8%
Omitting Pouryazdanparast, 2012     34.7%
Omitting Brown, 2012                30.4%
Omitting Menzies, 2012              43.8%

Pooled estimate                     38.0%
```

```
Details on meta-analytical method:
- Inverse variance method
- DerSimonian-Laird estimator for tau^2
```

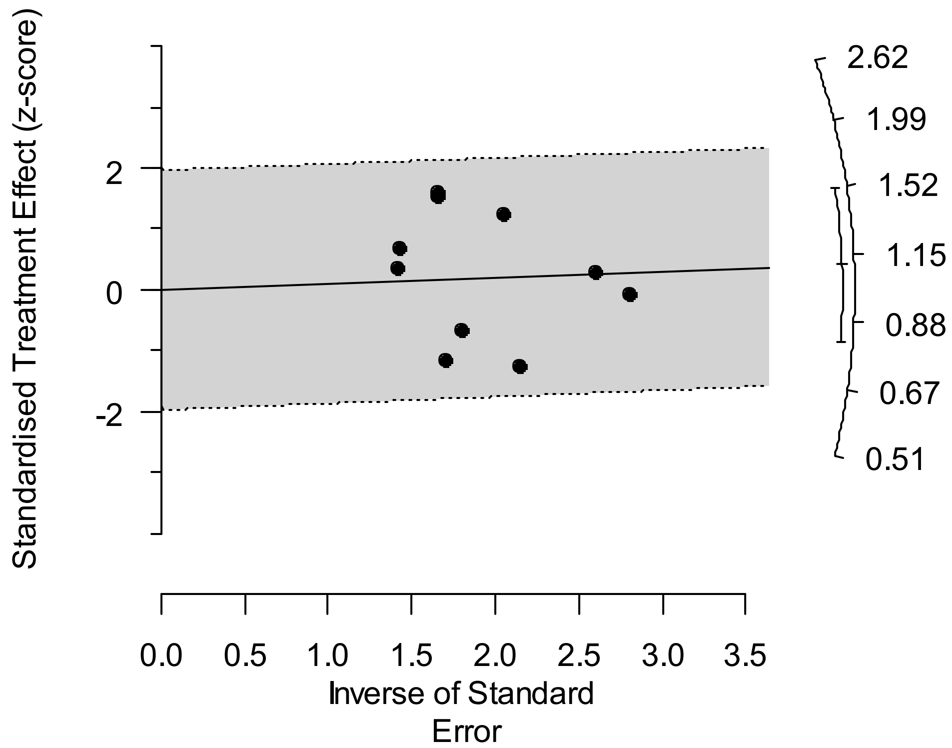



| | HR | 95%-CI | %W(fixed) | %W(random) |
|---|---|---|---|---|
| Broekaert, 2010 | 1.1235 | [0.6953; 1.8152] | 19.79 | 13.48 |
| Devitt, 2011 | 1.8200 | [0.8541; 3.8784] | 7.96 | 9.53 |
| Shinozaki, 2004 | 2.5391 | [0.9063; 7.1138] | 4.29 | 6.72 |
| Deichmann, 2004 | 0.6942 | [0.2749; 1.7531] | 5.31 | 7.66 |
| Pouryazdanparast, 2012 | 0.5088 | [0.1883; 1.3749] | 4.61 | 7.03 |
| Brown, 2012 | 2.6200 | [0.9357; 7.3360] | 4.30 | 6.72 |
| Menzies, 2012 | 0.9799 | [0.6642; 1.4457] | 30.12 | 14.91 |
| Takata, 2007 | 1.6000 | [0.4625; 5.5348] | 2.96 | 5.21 |
| Casula, 2004 | 1.2967 | [0.3679; 4.5698] | 2.87 | 5.10 |
| Kumar, 2003 | 0.5571 | [0.2758; 1.1253] | 9.22 | 10.21 |
| Filled: Shinozaki, 2004 | 0.3766 | [0.1344; 1.0552] | 4.29 | 6.72 |

```
Filled: Brown, 2012     0.3650  [0.1304; 1.0220]      4.30       6.72

Number of studies combined: k=12 (with 2 added studies)

                        HR            95%-CI      z  p.value
Fixed effect model   0.9779  [0.7900; 1.2106] -0.205   0.8376
Random effects model 0.9669  [0.6927; 1.3496] -0.198   0.843

Quantifying heterogeneity:
tau^2 = 0.1548; H = 1.41 [1.01; 1.97]; I^2 = 50% [2.9%; 74.2%]

Test of heterogeneity:
     Q d.f.  p.value
 21.98   11   0.0245

Details on meta-analytical method:
- Inverse variance method
- DerSimonian-Laird estimator for tau^2
- Trim-and-fill method to adjust for funnel plot asymmetry
```



```
Regression Test for Funnel Plot Asymmetry

model:     mixed-effects meta-regression model
predictor: inverse of the total sample size

z = 1.7422, p = 0.0815
```



## One stage IPD data analysis for BRAF mutation

We finished above the two stage meta-analysis of the selected articles for the investigation of the prognostic value of BRAF gene in melanoma cases. The 1 st stage is considered the extraction of data, which in our case was from K-M survival curves, the Cox HRs from the study itself or new Cox HRs from the analysis of the IPD data of the study. We aim now to perform a one stage meta-analysis of the IPD data, stratified by study and using Cox proportional hazards. This is a fairly new and less used method but can give more insight into the variables of our data. The studies that were involved and their characteristics are in the tables below:

**Table 14: Information on included studies with BRAF for OS, MSS, DFS through IPD analysis - part 1.**

| Author | Year | Continent | Countries | only_met_patients | Specimen_Type | fixation | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Ugurel** | 2007 | Europe | Germany | 1 | metastatic | frozen | | | |
| **Caramuta** | 2010 | Europe | Sweden | 0 | metastatic | formalin | | | |
| **Pouryazdanparast** | 2012 | NorthA | USA | 0 | pri_cut | formalin | | | |
| **Lu** | 2012 | Asia | China | 0 | pri_cut | formalin | | | |
| **Akslen** | 2005 | Europe | Norway | 0 | pri_cut | formalin | | | |
| **Takata** | 2007 | Asia | Japan | 0 | pri_cut | formalin | | | |
| **Casula** | 2004 | Europe | Italy | 0 | pri_met | NA | | | |

**Table 15: Information on included studies with BRAF for OS, MSS, DFS through IPD analysis - part 2.**

| Author | Year | time_from | design | braf_exons | blinding | IOR | SOR | other_risk | |
|---|---|---|---|---|---|---|---|---|---|
| **Ugurel** | 2007 | metastatic | Prospective | 11_15 | Y | Low | Low | Low | |
| **Caramuta** | 2010 | metastatic | Retrospective | 15 | Y | Low | Low | Low | |
| **Pouryazdanparast** | 2012 | primary | Retrospective | 15 | Y | High | High | Low | |
| **Lu** | 2012 | primary | Retrospective | 11_15 | Y | Low | Low | Low | |
| **Akslen** | 2005 | primary | Retrospective | 15 | Y | Low | High | Low | |
| **Takata** | 2007 | primary | Retrospective | 15 | Y | Low | High | Low | |
| **Casula** | 2004 | primary | Retrospective | 3_15 | Y | High | High | High | |

**Table 16: Information on included studies with BRAF for OS, MSS, DFS through IPD analysis - part 3.**

| Author | Year | braf_n | braf_n_wt | braf_n_total | nras_n | nras_n_wt | nras_n_total | | |
|---|---|---|---|---|---|---|---|---|---|
| **Ugurel** | 2007 | 53 | 44 | 97 | 22 | 75 | 97 | | |
| **Caramuta** | 2010 | 12 | 20 | 32 | 9 | 23 | 32 | | |
| **Pouryazdanparast** | 2012 | 19 | 13 | 32 | 4 | 28 | 32 | | |
| **Lu** | 2012 | 106 | 289 | 395 | 29 | 366 | 395 | | |
| **Akslen** | 2005 | 15 | 36 | 51 | 14 | 38 | 52 | | |
| **Takata** | 2007 | 9 | 12 | 21 | 3 | 8 | 11 | | |
| **Casula** | 2004 | 11 | 1 | 12 | Not tested | Not tested | Not tested | | |

**Table 17: Information on included studies with BRAF for OS, MSS, DFS through IPD analysis - part 4.**

| Author | Year | hr_dfs_braf | hr_os_braf | hr_mss_braf | hr_osmss_braf | hr_dfs_nras | hr_os_nras | hr_mss_nras | hr_osmss_nras |
|---|---|---|---|---|---|---|---|---|---|
| **Ugurel** | 2007 | | 1.63 | | 1.63 | | 0.60 | | 0.60 |
| **Caramuta** | 2010 | | 0.94 | 1.35 | 0.94 | | 0.75 | 0.32 | 0.75 |
| **Pouryazdanparast** | 2012 | 0.51 | 0.25 | 0.25 | 0.25 | 1.86 | | 4.36 | 4.36 |
| **Lu** | 2012 | | 1.43 | | 1.43 | | 1.70 | | 1.70 |
| **Akslen** | 2005 | | 1.65 | 1.95 | 1.65 | | 1.19 | 0.88 | 1.19 |
| **Takata** | 2007 | 1.60 | 0.48 | | 0.48 | 0.19 | 4.07 | | 4.07 |
| **Casula** | 2004 | 1.30 | 0.86 | | 0.86 | | | | |

By the analysis below we see that there is no significant result for DFS or MSS. For both of those outcomes we suspect that a larger number is necessary for statistically significant results. Interestingly enough, for DFS there is a trend for the BRAF mutated melanomas to have longer DFS, something that has also been noticed by other researchers. However this is not statistically significant. There was also not difference when the data were grouped by stage.

### DFS BRAF

```
Call:

  n= 146, number of events= 103
   (576 observations deleted due to missingness)

         coef exp(coef) se(coef)     z Pr(>|z|)
BRAFwt 0.3112    1.3651   0.2503 1.243    0.214

       exp(coef) exp(-coef) lower .95 upper .95
BRAFwt     1.365     0.7326    0.8358     2.229

Concordance= 0.538  (se = 0.051 )
Rsquare= 0.01   (max possible= 0.979 )
Likelihood ratio test= 1.53  on 1 df,   p=0.2164
Wald test            = 1.55  on 1 df,   p=0.2137
Score (logrank) test = 1.55  on 1 df,   p=0.2128
```

### MSS BRAF

```
Call:

  n= 147, number of events= 65
   (575 observations deleted due to missingness)
```

```
          coef exp(coef) se(coef)      z Pr(>|z|)
BRAFwt -0.1887    0.8280   0.3444 -0.548    0.584

       exp(coef) exp(-coef) lower .95 upper .95
BRAFwt     0.828      1.208    0.4216     1.626

Concordance= 0.524  (se = 0.054 )
Rsquare= 0.002   (max possible= 0.928 )
Likelihood ratio test= 0.3  on 1 df,   p=0.5869
Wald test            = 0.3  on 1 df,   p=0.5837
Score (logrank) test = 0.3  on 1 df,   p=0.5834
```

However, there is statistical significance for OS. The HR=1.357, p-value=0.0119, meaning that BRAF mutated melanoma tumours have almost 36% increase risk of death compared to the wild-type.

### OS BRAF

```
Call:

  n= 683, number of events= 323
   (39 observations deleted due to missingness)

          coef exp(coef) se(coef)      z Pr(>|z|)
BRAFwt -0.3056    0.7367   0.1215 -2.516   0.0119 *
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

       exp(coef) exp(-coef) lower .95 upper .95
BRAFwt    0.7367      1.357    0.5807    0.9347

Concordance= 0.531  (se = 0.024 )
Rsquare= 0.009   (max possible= 0.98 )
Likelihood ratio test= 6.2  on 1 df,   p=0.01278
Wald test            = 6.33  on 1 df,   p=0.01187
Score (logrank) test = 6.37  on 1 df,   p=0.0116
```

However, now that we have IPD data we want to search more for known co-founders like sex and age, and also investigate stage and thickness of tumours. Interestingly, we see that stages III and IV are now statistically significant, whereas the BRAF mutation looses its significance. Sex and age are also not significant when combined with stage.

## OS BRAF + stage

```
Call:

  n= 600, number of events= 276
   (122 observations deleted due to missingness)

             coef exp(coef) se(coef)      z Pr(>|z|)
BRAFwt   -0.1278    0.8800   0.1344 -0.951   0.3417
StageII   0.7129    2.0399   0.5968  1.195   0.2323
StageIII  1.4007    4.0579   0.5927  2.363   0.0181 *
StageIV   2.5117   12.3255   0.5922  4.242 2.22e-05 ***
StageUN   0.7287    2.0723   0.7334  0.994   0.3204
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

         exp(coef) exp(-coef) lower .95 upper .95
BRAFwt       0.880    1.13636    0.6762     1.145
StageII      2.040    0.49023    0.6333     6.570
StageIII     4.058    0.24643    1.2701    12.965
StageIV     12.325    0.08113    3.8616    39.341
StageUN      2.072    0.48256    0.4923     8.724

Concordance= 0.753  (se = 0.028 )
Rsquare= 0.183   (max possible= 0.981 )
Likelihood ratio test= 121.3  on 5 df,   p=0
Wald test            = 122.2  on 5 df,   p=0
Score (logrank) test = 137.8  on 5 df,   p=0
```

## OS BRAF + Sex + Age + Stage

```
Call:

  n= 600, number of events= 276
   (122 observations deleted due to missingness)
```

```
             coef exp(coef)  se(coef)      z Pr(>|z|)    
BRAFwt   -0.154737  0.856640  0.136253 -1.136   0.2561    
SexM      0.112177  1.118710  0.123825  0.906   0.3650    
Age       0.004904  1.004916  0.004437  1.105   0.2690    
StageII   0.692413  1.998533  0.596992  1.160   0.2461    
StageIII  1.385390  3.996382  0.592786  2.337   0.0194 *  
StageIV   2.513470 12.347697  0.592389  4.243 2.21e-05 ***
StageUN   0.722153  2.058862  0.733912  0.984   0.3251    
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

         exp(coef) exp(-coef) lower .95 upper .95
BRAFwt      0.8566    1.16735    0.6559     1.119
SexM        1.1187    0.89389    0.8776     1.426
Age         1.0049    0.99511    0.9962     1.014
StageII     1.9985    0.50037    0.6202     6.440
StageIII    3.9964    0.25023    1.2505    12.772
StageIV    12.3477    0.08099    3.8667    39.430
StageUN     2.0589    0.48571    0.4886     8.676

Concordance= 0.758  (se = 0.029 )
Rsquare= 0.186   (max possible= 0.981 )
Likelihood ratio test= 123.5  on 7 df,   p=0
Wald test            = 123.6  on 7 df,   p=0
Score (logrank) test = 139.6  on 7 df,   p=0
```

## OS BRAF + Stage + BRAF*Stage

```
Call:

  n= 600, number of events= 276 
   (122 observations deleted due to missingness)

                      coef  exp(coef)   se(coef)      z Pr(>|z|)  
BRAFwt          -1.656e+00  1.909e-01  1.227e+00 -1.350   0.1770  
StageII         -1.014e-01  9.036e-01  7.592e-01 -0.134   0.8938  
StageIII         5.229e-01  1.687e+00  7.414e-01  0.705   0.4806  
StageIV          1.750e+00  5.753e+00  7.275e-01  2.405   0.0162 *
StageUN         -1.276e+01  2.866e-06  1.755e+03 -0.007   0.9942  
```

```
BRAFwt:StageII   1.562e+00  4.769e+00  1.264e+00  1.236   0.2165
BRAFwt:StageIII  1.668e+00  5.300e+00  1.256e+00  1.328   0.1841
BRAFwt:StageIV   1.486e+00  4.418e+00  1.241e+00  1.197   0.2312
BRAFwt:StageUN   1.425e+01  1.549e+06  1.755e+03  0.008   0.9935
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

                exp(coef) exp(-coef) lower .95 upper .95
BRAFwt          1.909e-01  5.238e+00   0.01725     2.113
StageII         9.036e-01  1.107e+00   0.20405     4.002
StageIII        1.687e+00  5.928e-01   0.39449     7.214
StageIV         5.753e+00  1.738e-01   1.38235    23.941
StageUN         2.866e-06  3.489e+05   0.00000       Inf
BRAFwt:StageII  4.769e+00  2.097e-01   0.40035    56.817
BRAFwt:StageIII 5.300e+00  1.887e-01   0.45240    62.080
BRAFwt:StageIV  4.418e+00  2.263e-01   0.38818    50.284
BRAFwt:StageUN  1.549e+06  6.455e-07   0.00000       Inf

Concordance= 0.747  (se = 0.028 )
Rsquare= 0.186   (max possible= 0.981 )
Likelihood ratio test= 123.5  on 9 df,   p=0
Wald test            = 121.4  on 9 df,   p=0
Score (logrank) test = 138.8  on 9 df,   p=0
```

## OS BRAF + Stage + Sex + Age + Thickness

```
Call:

  n= 394, number of events= 144
   (328 observations deleted due to missingness)

                          coef exp(coef)  se(coef)      z Pr(>|z|)
BRAFwt               -0.198841  0.819680  0.185882 -1.070  0.28475
StageII               0.518746  1.679920  0.625311  0.830  0.40678
StageIII              1.415689  4.119323  0.624378  2.267  0.02337 *
StageIV               2.497144 12.147746  0.614957  4.061 4.89e-05 ***
StageUN               0.782872  2.187747  0.756108  1.035  0.30048
SexM                 -0.025221  0.975095  0.176392 -0.143  0.88631
Age                   0.017524  1.017679  0.006722  2.607  0.00913 **
```

```
as.numeric(Thickness)  0.001109  1.001110  0.005777  0.192  0.84773
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

                      exp(coef) exp(-coef) lower .95 upper .95
BRAFwt                   0.8197    1.21999    0.5694     1.180
StageII                  1.6799    0.59527    0.4932     5.722
StageIII                 4.1193    0.24276    1.2116    14.005
StageIV                 12.1477    0.08232    3.6395    40.546
StageUN                  2.1877    0.45709    0.4970     9.629
SexM                     0.9751    1.02554    0.6901     1.378
Age                      1.0177    0.98263    1.0044     1.031
as.numeric(Thickness)    1.0011    0.99889    0.9898     1.013

Concordance= 0.781  (se = 0.03 )
Rsquare= 0.225   (max possible= 0.967 )
Likelihood ratio test= 100.2  on 8 df,   p=0
Wald test            = 101  on 8 df,   p=0
Score (logrank) test = 125.6  on 8 df,   p=0
```

Analysis of the prognostic value of BRAF per stage does not give any statistically significant results. That changes in a non-stratified model for stage IV only.

## OS BRAF for stage IV (stratified model)

```
Call:

  n= 183, number of events= 135
   (95 observations deleted due to missingness)

          coef exp(coef) se(coef)      z Pr(>|z|)
BRAFwt -0.2185    0.8037   0.1803 -1.212    0.226

       exp(coef) exp(-coef) lower .95 upper .95
BRAFwt    0.8037      1.244    0.5645     1.144

Concordance= 0.507  (se = 0.034 )
Rsquare= 0.008   (max possible= 0.995 )
Likelihood ratio test= 1.46  on 1 df,   p=0.2266
Wald test            = 1.47  on 1 df,   p=0.2255
Score (logrank) test = 1.47  on 1 df,   p=0.2247
```

## OS BRAF for stage IV (non-stratified model)

```
Call:

  n= 183, number of events= 135
   (95 observations deleted due to missingness)

          coef exp(coef) se(coef)      z Pr(>|z|)
BRAFwt -0.4273    0.6523   0.1738 -2.458    0.014 *
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

       exp(coef) exp(-coef) lower .95 upper .95
BRAFwt    0.6523      1.533    0.4639     0.917

Concordance= 0.567  (se = 0.024 )
Rsquare= 0.032   (max possible= 0.998 )
Likelihood ratio test= 5.94  on 1 df,   p=0.01482
Wald test            = 6.04  on 1 df,   p=0.01396
Score (logrank) test = 6.13  on 1 df,   p=0.01326
```

## OS BRAF + Age for stage IV (stratified model)

```
Call:

  n= 183, number of events= 135
   (95 observations deleted due to missingness)

            coef exp(coef)  se(coef)      z Pr(>|z|)
BRAFwt -0.197538  0.820749  0.182711 -1.081    0.280
Age    -0.004780  0.995231  0.006095 -0.784    0.433

       exp(coef) exp(-coef) lower .95 upper .95
BRAFwt    0.8207      1.218    0.5737     1.174
Age       0.9952      1.005    0.9834     1.007

Concordance= 0.504  (se = 0.042 )
Rsquare= 0.011   (max possible= 0.995 )
Likelihood ratio test= 2.08  on 2 df,   p=0.3538
Wald test            = 2.08  on 2 df,   p=0.3538
```

```
Score (logrank) test = 2.09  on 2 df,   p=0.3525
```

## OS BRAF + Age for stage IV (non-stratified model)

```
Call:

  n= 183, number of events= 135
   (95 observations deleted due to missingness)

            coef exp(coef)  se(coef)      z Pr(>|z|)
BRAFwt -0.416521  0.659336  0.175034 -2.380   0.0173 *
Age    -0.003127  0.996878  0.006051 -0.517   0.6053
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

       exp(coef) exp(-coef) lower .95 upper .95
BRAFwt    0.6593      1.517    0.4679    0.9292
Age       0.9969      1.003    0.9851    1.0088

Concordance= 0.569  (se = 0.029 )
Rsquare= 0.033   (max possible= 0.998 )
Likelihood ratio test= 6.21  on 2 df,   p=0.04493
Wald test            = 6.31  on 2 df,   p=0.0427
Score (logrank) test = 6.4  on 2 df,   p=0.04079
```

## OS BRAF + Age + Sex for stage IV (stratified model)

```
Call:

  n= 183, number of events= 135
   (95 observations deleted due to missingness)

            coef exp(coef)  se(coef)      z Pr(>|z|)
BRAFwt -0.181946  0.833647  0.182985 -0.994   0.3201
Age    -0.005799  0.994218  0.006003 -0.966   0.3340
SexM    0.370080  1.447851  0.181344  2.041   0.0413 *
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

       exp(coef) exp(-coef) lower .95 upper .95
BRAFwt    0.8336     1.1995    0.5824     1.193
Age       0.9942     1.0058    0.9826     1.006
```

```
SexM      1.4479     0.6907    1.0148     2.066

Concordance= 0.557  (se = 0.042 )
Rsquare= 0.034   (max possible= 0.995 )
Likelihood ratio test= 6.26  on 3 df,   p=0.0995
Wald test            = 6.26  on 3 df,   p=0.09959
Score (logrank) test = 6.31  on 3 df,   p=0.09759
```

**OS BRAF + Age + Sex for stage IV (non-stratified model)**

```
Call:

  n= 183, number of events= 135
   (95 observations deleted due to missingness)

            coef exp(coef)  se(coef)      z Pr(>|z|)
BRAFwt -0.397974  0.671679  0.175334 -2.270   0.0232 *
Age    -0.004796  0.995215  0.006022 -0.796   0.4258
SexM    0.405154  1.499533  0.177478  2.283   0.0224 *
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

       exp(coef) exp(-coef) lower .95 upper .95
BRAFwt    0.6717     1.4888    0.4763    0.9471
Age       0.9952     1.0048    0.9835    1.0070
SexM      1.4995     0.6669    1.0590    2.1234

Concordance= 0.594  (se = 0.029 )
Rsquare= 0.061   (max possible= 0.998 )
Likelihood ratio test= 11.43  on 3 df,   p=0.009618
Wald test            = 11.56  on 3 df,   p=0.009053
Score (logrank) test = 11.71  on 3 df,   p=0.008438
```

No statistical significance is found for any other stage in either random or fixed-effects analysis. The random-effects analysis follows:

## OS BRAF for stage III (stratified model)

```
Call:

  n= 158, number of events= 66
   (111 observations deleted due to missingness)

           coef exp(coef) se(coef)      z Pr(>|z|)
BRAFwt -0.07018   0.93223  0.27225 -0.258    0.797

       exp(coef) exp(-coef) lower .95 upper .95
BRAFwt    0.9322      1.073    0.5467      1.59

Concordance= 0.549  (se = 0.048 )
Rsquare= 0   (max possible= 0.925 )
Likelihood ratio test= 0.07  on 1 df,   p=0.7972
Wald test            = 0.07  on 1 df,   p=0.7966
Score (logrank) test = 0.07  on 1 df,   p=0.7966
```

## OS BRAF + Age for stage III (stratified model)

```
Call:

  n= 158, number of events= 66
   (111 observations deleted due to missingness)

            coef exp(coef)  se(coef)      z Pr(>|z|)
BRAFwt -0.251755  0.777435  0.287479 -0.876    0.381
Age     0.021390  1.021621  0.009542  2.242    0.025 *
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

       exp(coef) exp(-coef) lower .95 upper .95
BRAFwt    0.7774     1.2863    0.4425     1.366
Age       1.0216     0.9788    1.0027     1.041
```

```
Concordance= 0.592  (se = 0.06 )
Rsquare= 0.033   (max possible= 0.925 )
Likelihood ratio test= 5.34  on 2 df,   p=0.06928
Wald test            = 5.06  on 2 df,   p=0.07982
Score (logrank) test = 5.08  on 2 df,   p=0.07876
```

## OS BRAF + Age + Sex for stage III (stratified model)

```
Call:

  n= 158, number of events= 66
   (111 observations deleted due to missingness)

            coef exp(coef)  se(coef)      z Pr(>|z|)
BRAFwt -0.266303  0.766207  0.288365 -0.923   0.3558
Age     0.022692  1.022951  0.009561  2.373   0.0176 *
SexM   -0.322843  0.724088  0.257805 -1.252   0.2105
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

       exp(coef) exp(-coef) lower .95 upper .95
BRAFwt    0.7662     1.3051    0.4354     1.348
Age       1.0230     0.9776    1.0040     1.042
SexM      0.7241     1.3810    0.4369     1.200

Concordance= 0.607  (se = 0.06 )
Rsquare= 0.043   (max possible= 0.925 )
Likelihood ratio test= 6.92  on 3 df,   p=0.07465
Wald test            = 6.65  on 3 df,   p=0.08385
Score (logrank) test = 6.72  on 3 df,   p=0.08125
```

## OS BRAF for stage II (stratified model)

```
Call:

  n= 219, number of events= 67
   (84 observations deleted due to missingness)
```

```
          coef exp(coef) se(coef)      z Pr(>|z|)
BRAFwt -0.2115    0.8094   0.3193 -0.662    0.508

       exp(coef) exp(-coef) lower .95 upper .95
BRAFwt    0.8094      1.236    0.4329     1.513

Concordance= 0.506  (se = 0.051 )
Rsquare= 0.002   (max possible= 0.841 )
Likelihood ratio test= 0.43  on 1 df,   p=0.5121
Wald test            = 0.44  on 1 df,   p=0.5077
Score (logrank) test = 0.44  on 1 df,   p=0.5072
```

**OS BRAF for stage I (stratified model)**

```
Call:

  n= 23, number of events= 3
   (84 observations deleted due to missingness)

          coef exp(coef) se(coef)      z Pr(>|z|)
BRAFwt -1.3268    0.2653   1.2257 -1.082    0.279

       exp(coef) exp(-coef) lower .95 upper .95
BRAFwt    0.2653      3.769   0.02401     2.932

Concordance= 0.519  (se = 0.181 )
Rsquare= 0.054   (max possible= 0.41 )
Likelihood ratio test= 1.27  on 1 df,   p=0.2603
Wald test            = 1.17  on 1 df,   p=0.2791
Score (logrank) test = 1.35  on 1 df,   p=0.2448
```

When BRAF is combined with Thickness of tumour it becomes statistically significant, but this is lost once we stratify by stage.

### OS BRAF + Thickness (stratified model)

```
Call:

  n= 474, number of events= 190
   (248 observations deleted due to missingness)

                          coef exp(coef)  se(coef)      z Pr(>|z|)
BRAFwt               -0.331280  0.718004  0.152614 -2.171   0.0300 *
as.numeric(Thickness)  0.007917  1.007949  0.004768  1.661   0.0968 .
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

                      exp(coef) exp(-coef) lower .95 upper .95
BRAFwt                    0.718     1.3928    0.5324    0.9683
as.numeric(Thickness)     1.008     0.9921    0.9986    1.0174

Concordance= 0.54  (se = 0.032 )
Rsquare= 0.017   (max possible= 0.968 )
Likelihood ratio test= 8.08  on 2 df,   p=0.01761
Wald test            = 8.2  on 2 df,   p=0.01657
Score (logrank) test = 8.26  on 2 df,   p=0.01606
```

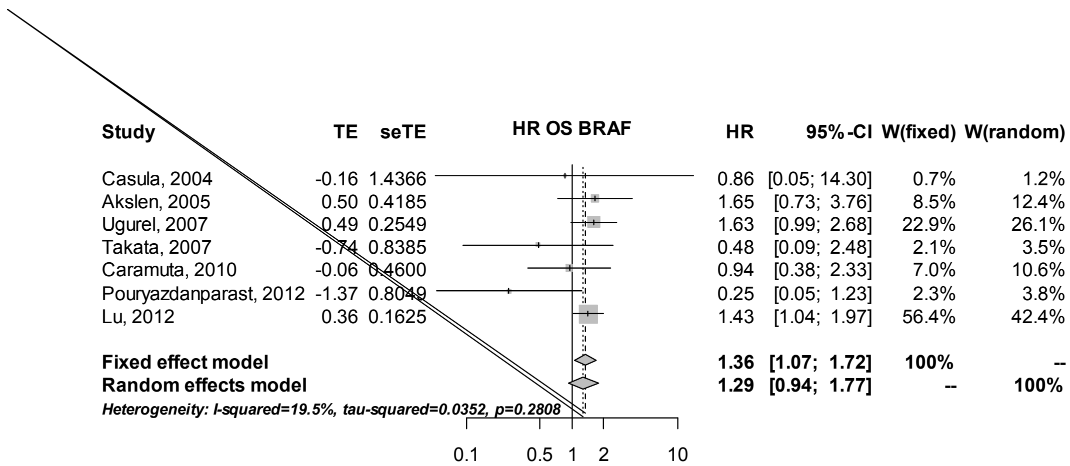
### OS BRAF + Thickness + Stage (stratified model)

```
Call:

  n= 394, number of events= 144
   (328 observations deleted due to missingness)

                           coef  exp(coef)   se(coef)      z Pr(>|z|)
BRAFwt               -0.1622360  0.8502405  0.1841868 -0.881   0.3784
as.numeric(Thickness)  0.0006431  1.0006433  0.0057451  0.112   0.9109
StageII                0.5664851  1.7620626  0.6249754  0.906   0.3647
StageIII               1.4132048  4.1091032  0.6252246  2.260   0.0238 *
StageIV                2.5083274 12.2843657  0.6139762  4.085  4.4e-05 ***
StageUN                0.7545225  2.1265958  0.7586388  0.995   0.3199
```

```
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

                      exp(coef) exp(-coef) lower .95 upper .95
BRAFwt                   0.8502     1.1761    0.5926     1.220
as.numeric(Thickness)    1.0006     0.9994    0.9894     1.012
StageII                  1.7621     0.5675    0.5177     5.998
StageIII                 4.1091     0.2434    1.2066    13.994
StageIV                 12.2844     0.0814    3.6875    40.923
StageUN                  2.1266     0.4702    0.4808     9.407

Concordance= 0.771  (se = 0.03 )
Rsquare= 0.211   (max possible= 0.967 )
Likelihood ratio test= 93.29  on 6 df,   p=0
Wald test            = 95  on 6 df,   p=0
Score (logrank) test = 118.9  on 6 df,   p=0
```

As part of a sensitivity analysis we performed the same meta-analysis using a typical random-effects model for the same studies that were included above and not a stratified Cox model. We also wanted to investigate the heterogeneity between the studies.

```
Review:     HR_OS for BRAF
                          TE seTE
Casula, 2004           -0.16 1.44
Akslen, 2005            0.50 0.42
Ugurel, 2007            0.49 0.25
Takata, 2007           -0.74 0.84
Caramuta, 2010         -0.06 0.46
Pouryazdanparast, 2012 -1.37 0.80
Lu, 2012                0.36 0.16


                         HR         95%-CI %W(fixed) %W(random)
Casula, 2004           0.86  [0.05; 14.30]      0.72       1.24
Akslen, 2005           1.65  [0.73;  3.76]      8.50      12.41
Ugurel, 2007           1.63  [0.99;  2.68]     22.92      26.05
Takata, 2007           0.48  [0.09;  2.48]      2.12       3.53
Caramuta, 2010         0.94  [0.38;  2.33]      7.04      10.57
Pouryazdanparast, 2012 0.25  [0.05;  1.23]      2.30       3.82
Lu, 2012               1.43  [1.04;  1.97]     56.40      42.37

Number of studies combined: k=7

                       HR        95%-CI    z  p.value
Fixed effect model   1.36  [1.07; 1.72] 2.50   0.0125
Random effects model 1.29  [0.94; 1.77] 1.58   0.1143

Quantifying heterogeneity:
tau^2 = 0.0352; H = 1.11 [1; 1.65]; I^2 = 19.5% [0%; 63.1%]

Test of heterogeneity:
    Q d.f.  p.value
 7.45    6   0.2808

Details on meta-analytical method:
- Inverse variance method
- DerSimonian-Laird estimator for tau^2
```



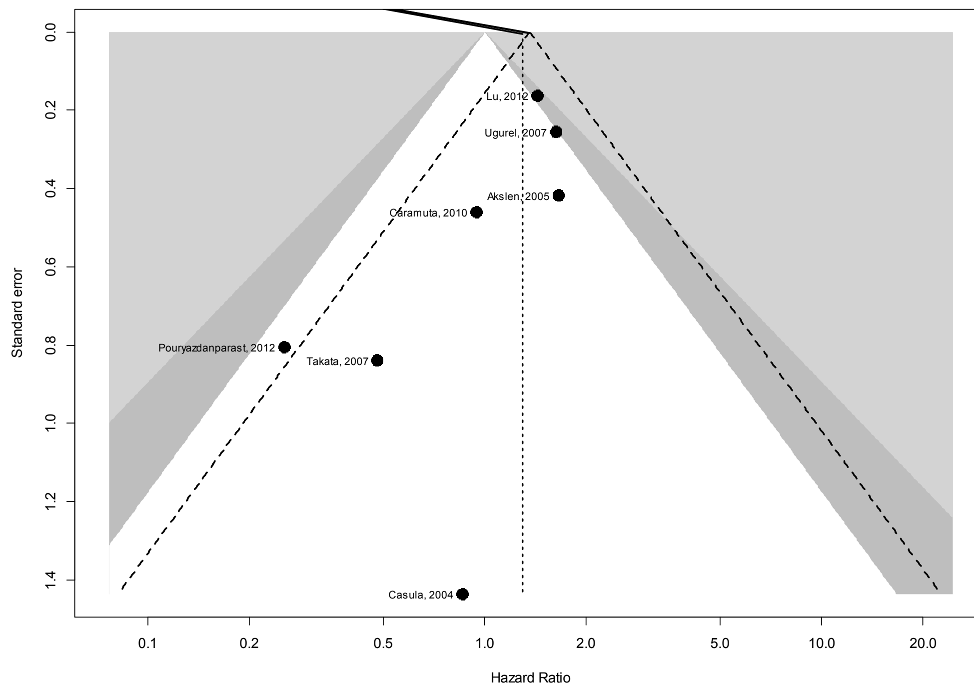

The results were almost identical but there doesn't seem to be any significant heterogeneity between the studies. Egger's and Peters tests are also non-significant. The radial plot does not show any heterogeneity, but the funnel plot suggests that we may be missing small studies with large HRs.

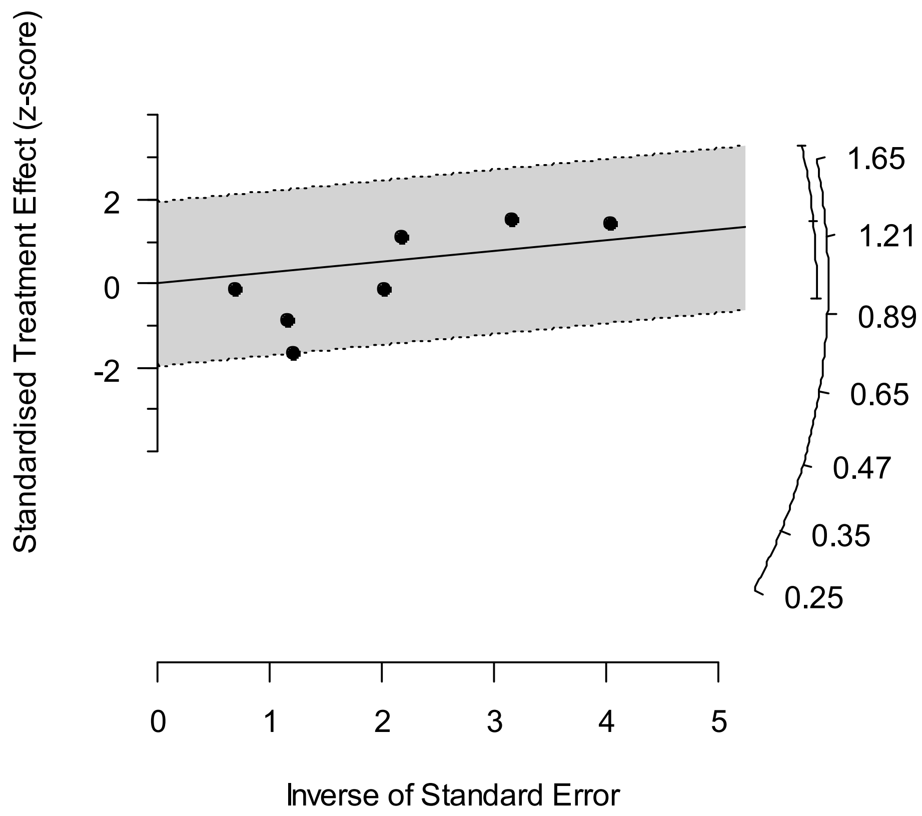
## Egger's test:

```
Review:     HR_OS for BRAF

        Linear regression test of funnel plot asymmetry

data:  brafosipd.meta
t = -2.2111, df = 5, p-value = 0.078
alternative hypothesis: asymmetry in funnel plot
sample estimates:
      bias    se.bias      slope
-1.2668212  0.5729457  0.6400465
```

## Peters test:

```
Regression Test for Funnel Plot Asymmetry

model:     mixed-effects meta-regression model
predictor: inverse of the total sample size

z = 0.9165, p = 0.3594
```




Standardised Treatment Effect (z-score)
2
0
-2
1.65
1.21
0.89
0.65
0.47
0.35
0.25
0
1
2
3
4
5
Inverse of Standard Error

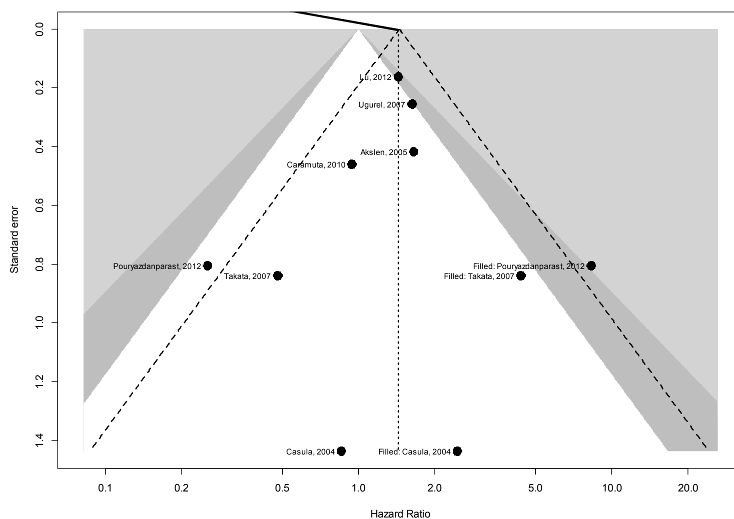
By an influential meta-analysis it seems that the study by Pouryazdanparast et al. (2012) is the most different to the other 6 studies.

```
Influential analysis (Random effects model)

                                     HR            95%-CI  p.value    tau^2
Omitting Casula, 2004            1.2665  [0.8895; 1.8033]   0.1901   0.0578
Omitting Akslen, 2005            1.1936  [0.8088; 1.7615]   0.3729   0.0653
Omitting Ugurel, 2007            1.1222  [0.7242; 1.7387]   0.606    0.0786
Omitting Takata, 2007            1.3543  [1.0054; 1.8244]   0.046    0.0223
Omitting Caramuta, 2010          1.3174  [0.9165; 1.8936]   0.1365   0.051
Omitting Pouryazdanparast, 2012  1.4109  [1.1077; 1.7972]   0.0053   0
Omitting Lu, 2012                1.1044  [0.6668; 1.8293]   0.6997   0.1145

Pooled estimate                  1.2905  [0.9403; 1.7711]   0.1143   0.0352
                                    I^2
Omitting Casula, 2004             32.0%
Omitting Akslen, 2005             30.6%
Omitting Ugurel, 2007             26.5%
Omitting Takata, 2007             15.0%
Omitting Caramuta, 2010           26.4%
Omitting Pouryazdanparast, 2012    0.0%
Omitting Lu, 2012                 30.5%

Pooled estimate                   19.5%

Details on meta-analytical method:
- Inverse variance method
- DerSimonian-Laird estimator for tau^2
```

A trim and fill analysis adds 3 studies, increasing the heterogeneity, but not significantly and increasing the significance of the results.

```
                                 HR            95%-CI %W(fixed) %W(random)
Ugurel, 2007                 1.6256  [0.9864;  2.6791]     21.80      22.09
Caramuta, 2010               0.9445  [0.3834;  2.3268]      6.69      12.21
Pouryazdanparast, 2012       0.2532  [0.0523;  1.2263]      2.19       5.24
Lu, 2012                     1.4329  [1.0421;  1.9703]     53.64      28.06
```

```
Akslen, 2005                    1.6547  [0.7286;  3.7578]      8.09      13.73
Takata, 2007                    0.4790  [0.0926;  2.4779]      2.01       4.88
Casula, 2004                    0.8563  [0.0513; 14.3046]      0.69       1.84
Filled: Casula, 2004            2.4582  [0.1472; 41.0637]      0.69       1.84
Filled: Takata, 2007            4.3944  [0.8495; 22.7319]      2.01       4.88
Filled: Pouryazdanparast, 2012 8.3140  [1.7166; 40.2664]      2.19       5.24

Number of studies combined: k=10 (with 3 added studies)

                         HR            95%-CI      z  p.value
Fixed effect model   1.4509  [1.1490; 1.8321] 3.1271   0.0018
Random effects model 1.4325  [0.9679; 2.1201] 1.7971   0.0723

Quantifying heterogeneity:
tau^2 = 0.1162; H = 1.26 [1; 1.83]; I^2 = 37.3% [0%; 70.1%]

Test of heterogeneity:
     Q d.f.  p.value
 14.35    9   0.1106

Details on meta-analytical method:
- Inverse variance method
- DerSimonian-Laird estimator for tau^2
- Trim-and-fill method to adjust for funnel plot asymmetry
```



Finally we wanted to see if indeed the percentage of BRAF mutated tumours increases from the lower to the higher stages. We see from the table below that indeed that is the case and a chi-squared test confirms that this is statistically significant.

```
BRAF and wt per stage

    BRAF  wt
I      7  16
II    58 161
III   75 110
IV    84 110


        Pearson's Chi-squared test

data:  brafipdsum
X-squared = 15.0435, df = 3, p-value = 0.00178


        Fisher's Exact Test for Count Data

data:  brafipdsum
p-value = 0.001525
alternative hypothesis: two.sided
```

## Outcomes of patients with NRAS and wild type (WT) mutations

The results are not statistically significant for the NRAS mutated tumours for either OS, DFS, MSS or OS and MSS combined. Below are the tables with their included studies and their characteristics and the analysis for every outcome measure. One stage IPD analysis was also performed, as for BRAF, but again did not reveal any statistically significant result. The studies below were used in various combination. Not all the studies were used in any one combination. Also some studies were used for sensitivity analysis, such as by Omhold or Demunter.

**Table 18: Information on included studies with NRAS for OS, MSS, DFS through IPD analysis - part 1.**

| Author | Year | Continent | Countries | data_from | only_met_patients | Specimen_Type | fixation |
|---|---|---|---|---|---|---|---|
| **Devitt** | 2011 | Oceania | Australia | COX_ARTICLE | 0 | pri_cut | formalin |
| **Omholt** | 2003 | Europe | Sweden | PVALUE_X2 | 1 | pri_cut | formalin |
| **Edlundh-Rose** | 2006 | Europe | Sweden | KM | 1 | pri_met | form_froz |
| **Ugurel** | 2007 | Europe | Germany | IPD | 1 | metastatic | frozen |
| **Caramuta** | 2010 | Europe | Sweden | IPD | 0 | metastatic | formalin |
| **Elsas** | 1996 | Eu_Af_Oc | Aus_Nethe_Afr | KM | 0 | pri_cut | formalin |
| **Omholt** | 2002 | Europe | Sweden | PVALUE_X2 | 1 | pri_cut | formalin |
| **Demunter** | 2001 | Europe | Belgium | PVALUE_X2 | 0 | pri_cut | form_froz |
| **Pouryazdanparast** | 2012 | NorthA | USA | IPD | 0 | pri_cut | formalin |
| **Jacob** | 2011 | NorthA | USA | KM | 1 | metastatic | NA |
| **Lu** | 2012 | Asia | China | IPD | 0 | pri_cut | formalin |
| **Akslen** | 2005 | Europe | Norway | IPD | 0 | pri_cut | formalin |
| **Turri** | 2012 | Europe | Italy | IPD | 0 | sinonasal | formalin |
| **Takata** | 2007 | Asia | Japan | IPD | 0 | pri_cut | formalin |
| **Kumar** | 2003 | Europe | Sweden | IPD | 1 | pri_met | frozen |

**Table 19: Information on included studies with NRAS for OS, MSS, DFS through IPD analysis - part 2.**

| Author | Year | time_from | design | blinding | IOR | SOR | other_risk |
|---|---|---|---|---|---|---|---|
| **Devitt** | 2011 | primary | Prospective | Y | High | High | High |
| **Omholt** | 2003 | primary | Retrospective | Y | High | High | High |
| **Edlundh-Rose** | 2006 | primary | Retrospective | Y | High | High | High |
| **Ugurel** | 2007 | metastatic | Prospective | Y | Low | Low | Low |
| **Caramuta** | 2010 | metastatic | Retrospective | Y | Low | Low | Low |
| **Elsas** | 1996 | metastatic | Retrospective | Y | High | High | High |
| **Omholt** | 2002 | primary | Retrospective | Y | High | Low | High |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **Demunter** | 2001 | primary | Retrospective | Y | Low | Low | Low |
| **Pouryazdanparast** | 2012 | primary | Retrospective | Y | High | High | Low |
| **Jacob** | 2011 | metastatic | Retrospective | Y | Low | Low | High |
| **Lu** | 2012 | primary | Retrospective | Y | Low | Low | Low |
| **Akslen** | 2005 | primary | Retrospective | Y | Low | High | Low |
| **Turri** | 2012 | primary | Retrospective | Y | Low | Low | Low |
| **Takata** | 2007 | primary | Retrospective | Y | Low | High | Low |
| **Kumar** | 2003 | primary | Retrospective | Y | Low | Low | Low |

**Table 20: Information on included studies with NRAS for OS, MSS, DFS through IPD analysis - part 3.**

| Author | nras_n | nras_n_wt | nras_n_total | hr_dfs_nras | hr_os_nras | hr_mss_nras | hr_osmss_nras |
|---|---|---|---|---|---|---|---|
| **Devitt** | 36 | 213 | 249 | 1.94 | 1.45 | 2.46 | 1.45 |
| **Omholt** | 21 | 50 | 71 | | 0.93 | | 0.93 |
| **Edlundh-Rose** | 61 | 158 | 219 | | 0.71 | | 0.71 |
| **Ugurel** | 22 | 75 | 97 | | 0.60 | | 0.60 |
| **Caramuta** | 9 | 23 | 32 | | 0.75 | 0.32 | 0.75 |
| **Elsas** | 42 | 228 | 270 | | 0.92 | | 0.92 |
| **Omholt** | 21 | 52 | 73 | | 0.88 | | 0.88 |
| **Demunter** | 16 | 35 | 51 | 1.14 | 7.99 | | 7.99 |
| **Pouryazdanparast** | 4 | 28 | 32 | 1.86 | | 4.36 | 4.36 |
| **Jacob** | 66 | 94 | 160 | | 2.05 | | 2.05 |
| **Lu** | 29 | 366 | 395 | | 1.70 | | 1.70 |
| **Akslen** | 14 | 38 | 52 | | 1.19 | 0.88 | 1.19 |
| **Turri** | 7 | 25 | 32 | 0.96 | 0.65 | 0.65 | 0.65 |
| **Takata** | 3 | 8 | 11 | 0.19 | 4.07 | | 4.07 |
| **Kumar** | 3 | 35 | 38 | 1.26 | | | |

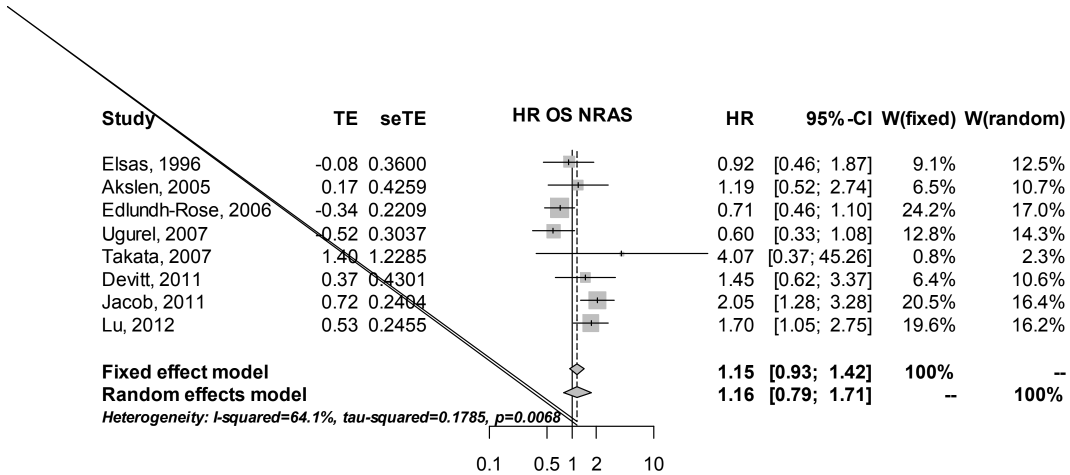
## OS NRAS

## Analysis

```
Review:     HR_OS for
NRAS
                     TE seTE
Elsas, 1996        -0.08 0.36
Akslen, 2005        0.17 0.43
Edlundh-Rose, 2006 -0.34 0.22
Ugurel, 2007       -0.52 0.30
Takata, 2007        1.40 1.23
Devitt, 2011        0.37 0.43
Jacob, 2011         0.72 0.24
Lu, 2012            0.53 0.25


                     HR          95%-CI %W(fixed) %W(random)
Elsas, 1996        0.92  [0.46;  1.87]      9.13      12.54
Akslen, 2005       1.19  [0.52;  2.74]      6.52      10.74
Edlundh-Rose, 2006 0.71  [0.46;  1.10]     24.23      17.00
Ugurel, 2007       0.60  [0.33;  1.08]     12.83      14.27
Takata, 2007       4.07  [0.37; 45.26]      0.78       2.29
Devitt, 2011       1.45  [0.62;  3.37]      6.40      10.63
Jacob, 2011        2.05  [1.28;  3.28]     20.48      16.35
Lu, 2012           1.70  [1.05;  2.75]     19.63      16.18

Number of studies combined: k=8

                       HR         95%-CI    z  p.value
Fixed effect model   1.15  [0.93; 1.42] 1.29   0.1962
Random effects model 1.16  [0.79; 1.71] 0.77   0.4415

Quantifying heterogeneity:
tau^2 = 0.1785; H = 1.67 [1.14; 2.44]; I^2 = 64.1% [23.1%; 83.2%]

Test of heterogeneity:
     Q d.f.  p.value
 19.49    7   0.0068

Details on meta-analytical method:
- Inverse variance method
```

```
- DerSimonian-Laird estimator for tau^2
```

## Forest plot



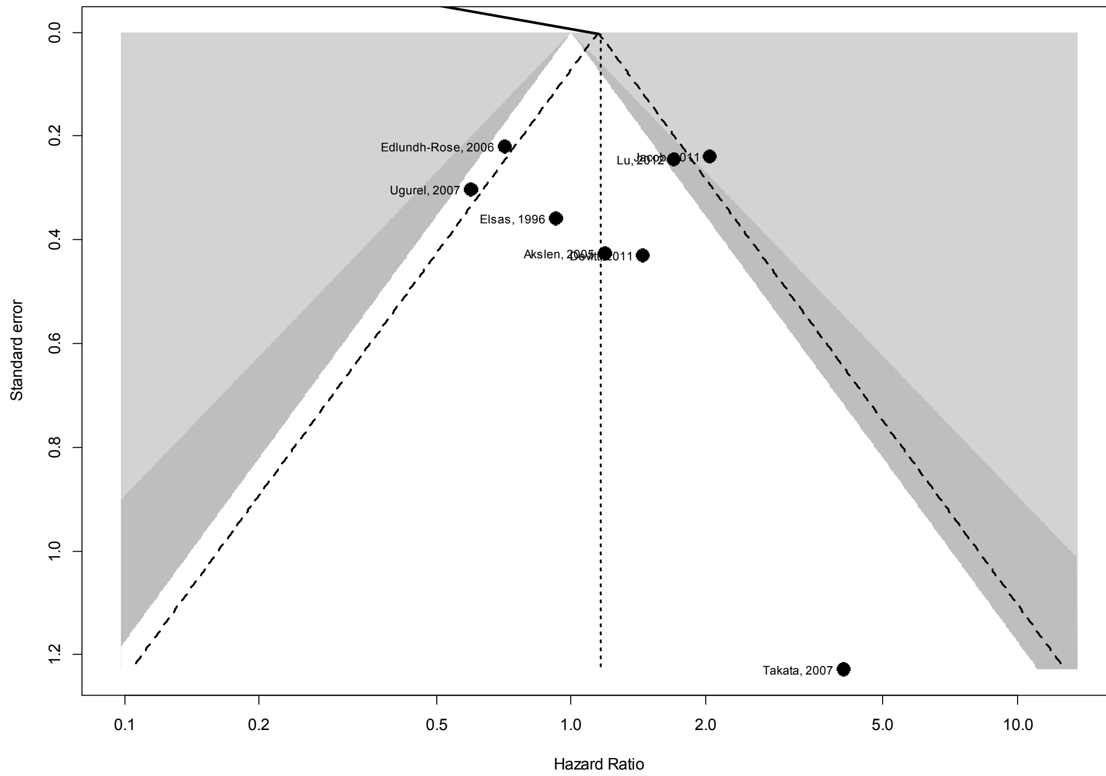

## Egger's test

```
Review:     HR_OS for
NRAS

        Linear regression test of funnel plot asymmetry

data:  nrasos.meta
t = 0.3724, df = 6, p-value = 0.7224
alternative hypothesis: asymmetry in funnel plot
sample estimates:
       bias     se.bias       slope
 0.66225563  1.77822660 -0.04993896
```

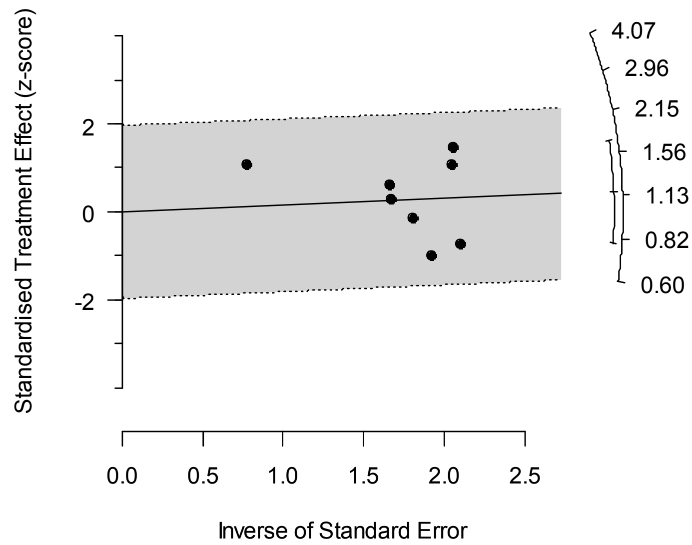
## Peter's test

```
Regression Test for Funnel Plot Asymmetry

model:     mixed-effects meta-regression model
predictor: inverse of the total sample size

z = -1.3275, p = 0.1844
```

## Funnel plot




## Radial plot

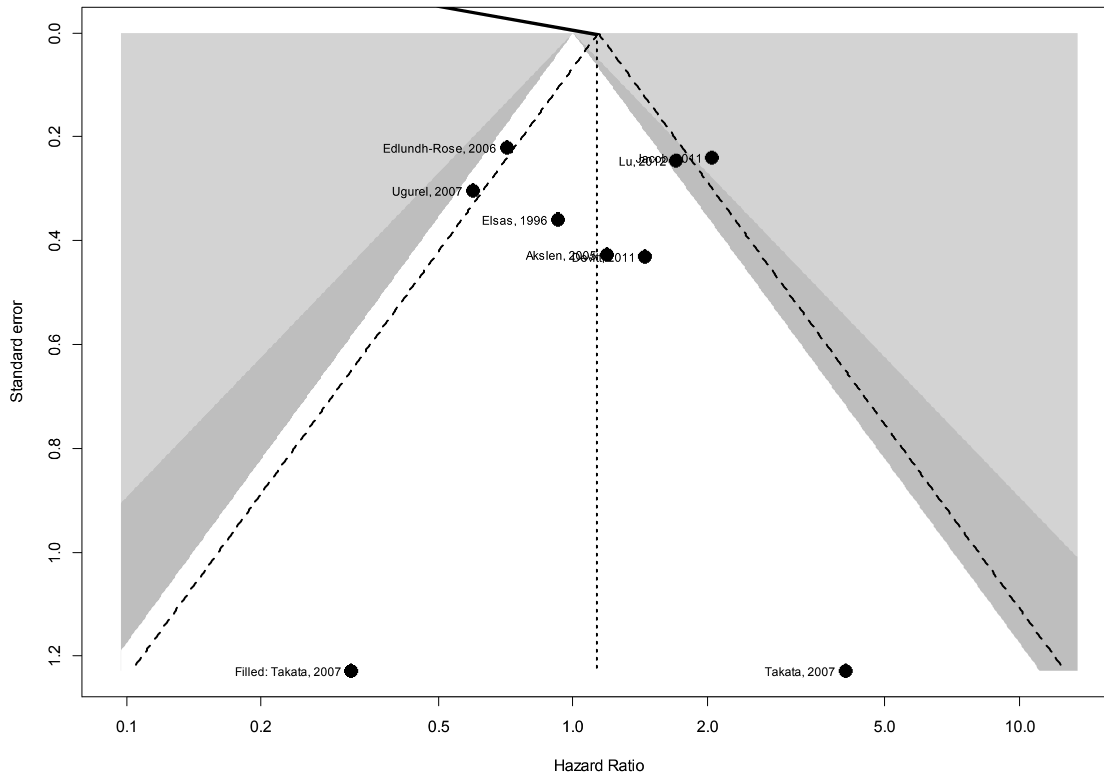


## Influential analysis

```
Influential analysis (Random effects model)

                                HR            95%-CI  p.value    tau^2
Omitting Elsas, 1996        1.2069  [0.7796; 1.8683]   0.3991   0.2124
Omitting Akslen, 2005       1.1637  [0.7554; 1.7926]   0.4916   0.2113
Omitting Edlundh-Rose, 2006 1.2873  [0.8687; 1.9077]   0.2082   0.1403
Omitting Ugurel, 2007       1.2958  [0.8827; 1.9023]   0.1859   0.1381
Omitting Takata, 2007       1.1296  [0.7644; 1.6693]   0.5406   0.1796
Omitting Devitt, 2011       1.1352  [0.7398; 1.7420]   0.5615   0.2062
Omitting Jacob, 2011        1.0292  [0.7097; 1.4926]   0.8792   0.1169
Omitting Lu, 2012           1.0832  [0.7040; 1.6666]   0.7162   0.1921

Pooled estimate             1.1633  [0.7914; 1.7101]   0.4415   0.1785
                                I^2
Omitting Elsas, 1996          68.5%
Omitting Akslen, 2005         69.2%
Omitting Edlundh-Rose, 2006   54.6%
Omitting Ugurel, 2007         57.4%
Omitting Takata, 2007         67.4%
Omitting Devitt, 2011         68.7%
Omitting Jacob, 2011          51.0%
Omitting Lu, 2012             63.3%

Pooled estimate               64.1%

Details on meta-analytical method:
- Inverse variance method
- DerSimonian-Laird estimator for tau^2
```

## Trim and Fill analysis

```
                        HR            95%-CI %W(fixed) %W(random)
Devitt, 2011        1.4500  [0.6242;  3.3685]      6.35      10.39
Edlundh-Rose, 2006  0.7107  [0.4609;  1.0959]     24.05      16.63
Ugurel, 2007        0.5955  [0.3284;  1.0800]     12.73      13.95
Elsas, 1996         0.9226  [0.4557;  1.8682]      9.06      12.26
Jacob, 2011         2.0500  [1.2799;  3.2836]     20.32      15.99
Lu, 2012            1.7001  [1.0508;  2.7507]     19.48      15.83
Akslen, 2005        1.1877  [0.5154;  2.7367]      6.47      10.49
Takata, 2007        4.0735  [0.3667; 45.2551]      0.78       2.23
Filled: Takata, 2007 0.3188  [0.0287;  3.5414]      0.78       2.23

Number of studies combined: k=9 (with 1 added studies)

                        HR           95%-CI      z  p.value
Fixed effect model   1.1395  [0.9215; 1.4091] 1.2054   0.228
Random effects model 1.1301  [0.7725; 1.6532] 0.6302   0.5286

Quantifying heterogeneity:
tau^2 = 0.1778; H = 1.6 [1.11; 2.31]; I^2 = 61.1% [19.4%; 81.2%]

Test of heterogeneity:
     Q d.f.  p.value
 20.57    8   0.0084

Details on meta-analytical method:
- Inverse variance method
- DerSimonian-Laird estimator for tau^2
- Trim-and-fill method to adjust for funnel plot asymmetry
```



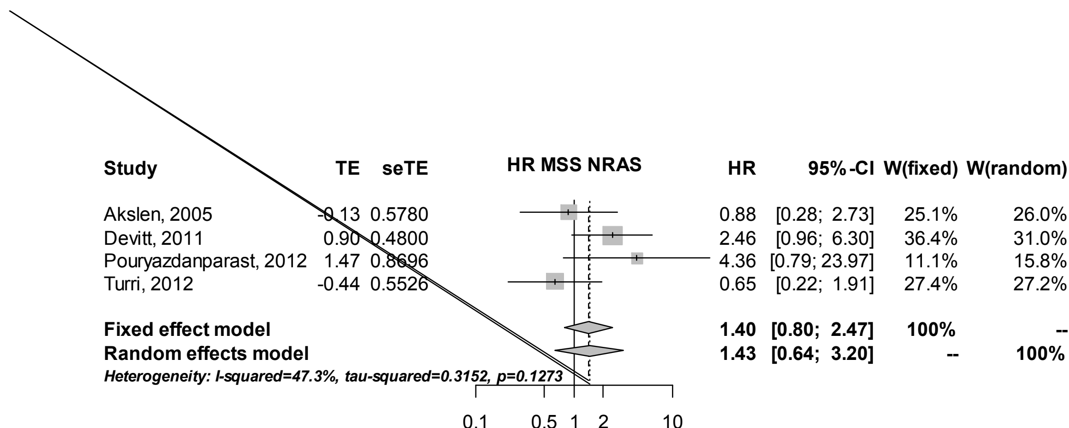

## MSS NRAS

## Analysis

```
Review:     HR_MSS for
NRAS
                          TE seTE
Akslen, 2005           -0.13 0.58
Devitt, 2011            0.90 0.48
Pouryazdanparast, 2012  1.47 0.87
Turri, 2012            -0.44 0.55


                         HR          95%-CI %W(fixed) %W(random)
Akslen, 2005           0.88  [0.28;  2.73]     25.09      26.03
Devitt, 2011           2.46  [0.96;  6.30]     36.38      30.97
```

```
Pouryazdanparast, 2012 4.36  [0.79; 23.97]     11.08      15.77
Turri, 2012            0.65  [0.22;  1.91]     27.45      27.23

Number of studies combined: k=4

                       HR        95%-CI    z  p.value
Fixed effect model   1.40  [0.80; 2.47] 1.17   0.2426
Random effects model 1.43  [0.64; 3.20] 0.87   0.3833

Quantifying heterogeneity:
tau^2 = 0.3152; H = 1.38 [1; 2.39]; I^2 = 47.3% [0%; 82.5%]

Test of heterogeneity:
   Q d.f.  p.value
 5.7    3   0.1273

Details on meta-analytical method:
- Inverse variance method
- DerSimonian-Laird estimator for tau^2
```

## Forest plot



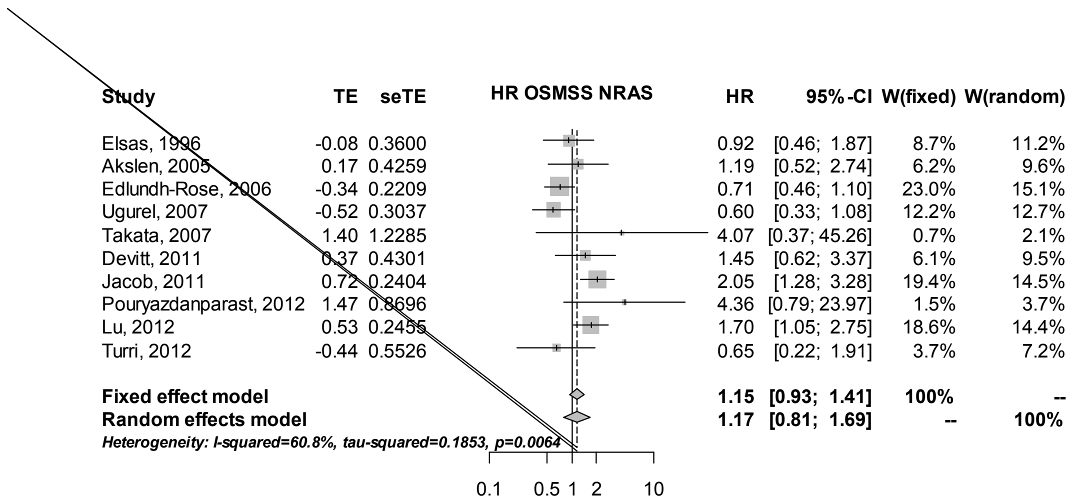
## OS and MSS NRAS

## Analysis

```
Review:     HR_osmss for NRAS
                            TE seTE
Elsas, 1996              -0.08 0.36
Akslen, 2005              0.17 0.43
Edlundh-Rose, 2006       -0.34 0.22
Ugurel, 2007             -0.52 0.30
Takata, 2007              1.40 1.23
Devitt, 2011              0.37 0.43
Jacob, 2011               0.72 0.24
Pouryazdanparast, 2012    1.47 0.87
Lu, 2012                  0.53 0.25
Turri, 2012              -0.44 0.55


                          HR         95%-CI %W(fixed) %W(random)
Elsas, 1996             0.92  [0.46;  1.87]      8.66      11.20
Akslen, 2005            1.19  [0.52;  2.74]      6.19       9.62
Edlundh-Rose, 2006      0.71  [0.46;  1.10]     22.98      15.06
Ugurel, 2007            0.60  [0.33;  1.08]     12.16      12.71
Takata, 2007            4.07  [0.37; 45.26]      0.74       2.08
Devitt, 2011            1.45  [0.62;  3.37]      6.07       9.53
Jacob, 2011             2.05  [1.28;  3.28]     19.42      14.51
Pouryazdanparast, 2012 4.36  [0.79; 23.97]      1.48       3.75
Lu, 2012                1.70  [1.05;  2.75]     18.62      14.36
Turri, 2012             0.65  [0.22;  1.91]      3.67       7.19

Number of studies combined: k=10

                        HR        95%-CI    z  p.value
Fixed effect model    1.15  [0.93; 1.41] 1.31   0.1892
Random effects model 1.17  [0.81; 1.69] 0.85   0.3975

Quantifying heterogeneity:
tau^2 = 0.1853; H = 1.6 [1.13; 2.26]; I^2 = 60.8% [21.6%; 80.4%]

Test of heterogeneity:
     Q d.f.  p.value
```

```
 22.93    9   0.0064

Details on meta-analytical method:
- Inverse variance method
- DerSimonian-Laird estimator for tau^2
```

## Forest plot



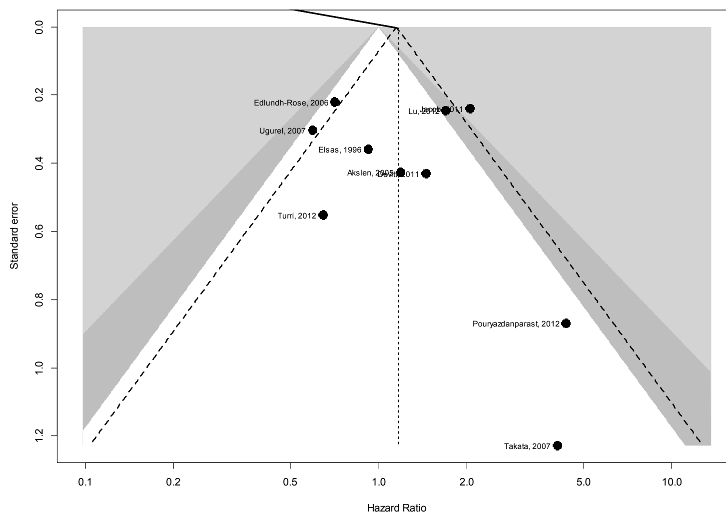
## Egger's test

```
Review:     HR_osmss for NRAS

        Linear regression test of funnel plot asymmetry

data:  nrasosmss.meta
t = 0.5576, df = 8, p-value = 0.5924
alternative hypothesis: asymmetry in funnel plot
sample estimates:
       bias     se.bias       slope 
 0.72070517  1.29254711 -0.08149254 
```

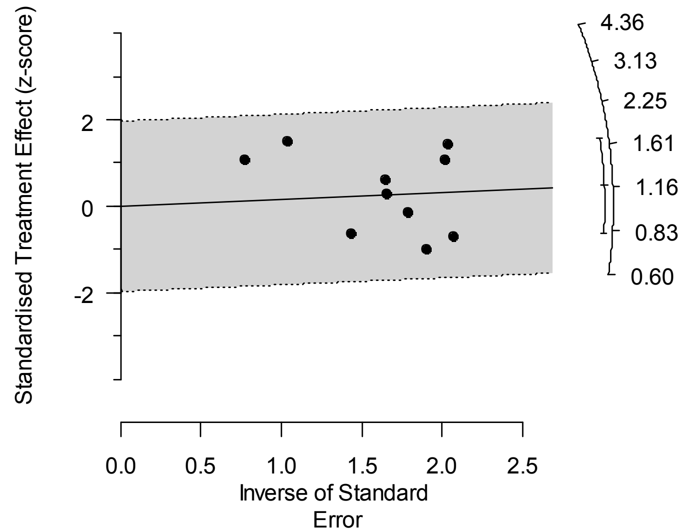
## Peters test

```
Regression Test for Funnel Plot Asymmetry

model:     mixed-effects meta-regression model
predictor: inverse of the total sample size

z = 1.4421, p = 0.1493
```

## Funnel plot



## Radial plot




## Influential analysis

```
Influential analysis (Random effects model)

                                   HR           95%-CI  p.value    tau^2
Omitting Elsas, 1996           1.2138  [0.8048; 1.8308]   0.3554   0.2172
Omitting Akslen, 2005          1.1756  [0.7826; 1.7659]   0.4358   0.216
Omitting Edlundh-Rose, 2006    1.2792  [0.8752; 1.8697]   0.2035   0.1552
Omitting Ugurel, 2007          1.2882  [0.8898; 1.8650]   0.1798   0.1502
Omitting Takata, 2007          1.1416  [0.7865; 1.6571]   0.4859   0.1863
Omitting Devitt, 2011          1.1500  [0.7680; 1.7219]   0.4974   0.2113
Omitting Jacob, 2011           1.0518  [0.7327; 1.5099]   0.7841   0.1319
Omitting Pouryazdanparast, 2012   1.1136  [0.7728; 1.6047]   0.5636   0.1703
Omitting Lu, 2012              1.1044  [0.7354; 1.6588]   0.6321   0.1995
Omitting Turri, 2012           1.2296  [0.8349; 1.8109]   0.2954   0.193

Pooled estimate                1.1722  [0.8112; 1.6938]   0.3975   0.1853
                                   I^2
Omitting Elsas, 1996             64.5%
Omitting Akslen, 2005            65.1%
Omitting Edlundh-Rose, 2006      52.4%
```

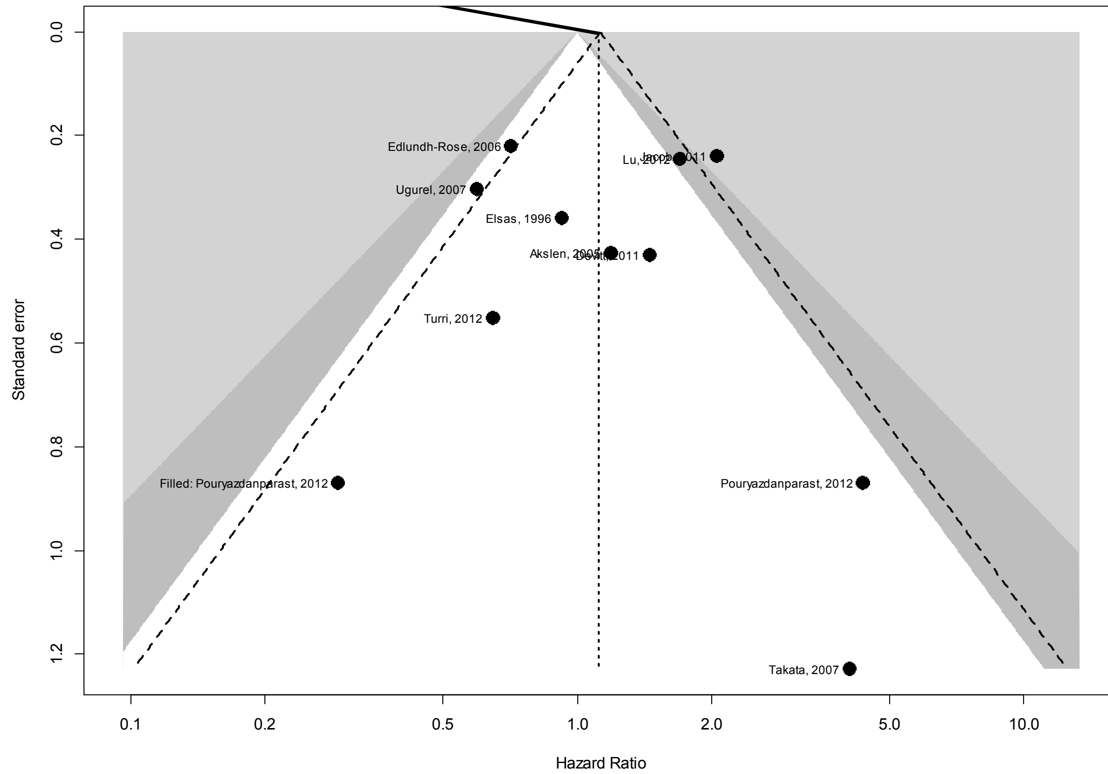
```
Omitting Ugurel, 2007             54.5%
Omitting Takata, 2007             63.4%
Omitting Devitt, 2011             64.6%
Omitting Jacob, 2011              49.2%
Omitting Pouryazdanparast, 2012   61.1%
Omitting Lu, 2012                 59.6%
Omitting Turri, 2012              63.3%

Pooled estimate                   60.8%

Details on meta-analytical method:
- Inverse variance method
- DerSimonian-Laird estimator for tau^2
```

## Trim and Fill analysis and plot

```
                                HR            95%-CI %W(fixed) %W(random)
Devitt, 2011                1.4500  [0.6242;  3.3685]     5.98       9.26
Edlundh-Rose, 2006          0.7107  [0.4609;  1.0959]    22.65      14.31
Ugurel, 2007                0.5955  [0.3284;  1.0800]    11.99      12.19
Elsas, 1996                 0.9226  [0.4557;  1.8682]     8.53      10.81
Pouryazdanparast, 2012      4.3601  [0.7930; 23.9720]     1.46       3.73
Jacob, 2011                 2.0500  [1.2799;  3.2836]    19.14      13.81
Lu, 2012                    1.7001  [1.0508;  2.7507]    18.34      13.68
Akslen, 2005                1.1877  [0.5154;  2.7367]     6.10       9.34
Turri, 2012                 0.6453  [0.2185;  1.9059]     3.62       7.05
Takata, 2007                4.0735  [0.3667; 45.2551]     0.73       2.09
Filled: Pouryazdanparast, 2012 0.2910  [0.0529;  1.5998]     1.46       3.73

Number of studies combined: k=11 (with 1 added studies)

                         HR           95%-CI      z  p.value
Fixed effect model   1.1264  [0.9166; 1.3842] 1.1318   0.2577
Random effects model 1.1149  [0.7699; 1.6147] 0.5757   0.5648

Quantifying heterogeneity:
tau^2 = 0.2007; H = 1.59 [1.15; 2.22]; I^2 = 60.6% [23.8%; 79.6%]

Test of heterogeneity:
     Q d.f.  p.value
 25.39   10   0.0047
```

Details on meta-analytical method:

- Inverse variance method
- DerSimonian-Laird estimator for tau^2
- Trim-and-fill method to adjust for funnel plot asymmetry

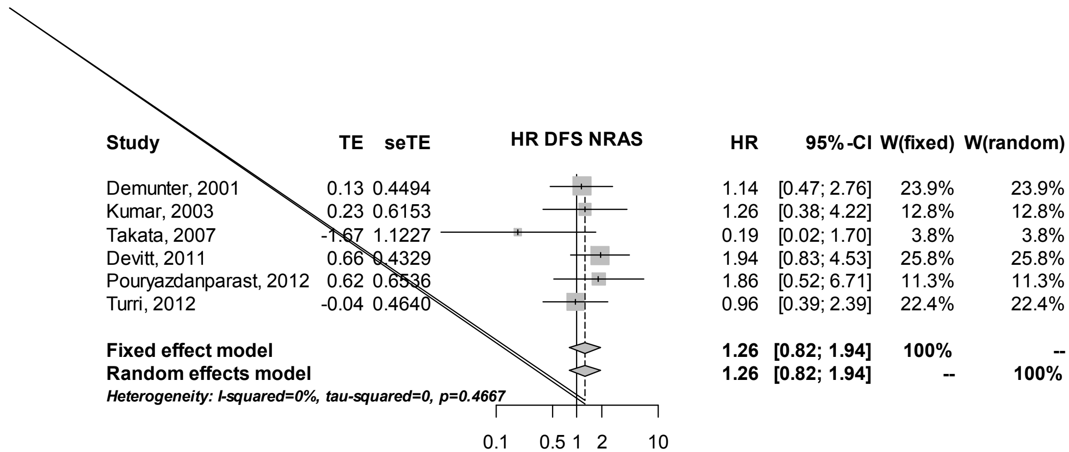


## DFS NRAS

## Analysis

```
Review:     HR_DFS for
NRAS
                          TE seTE
Demunter, 2001          0.13 0.45
Kumar, 2003             0.23 0.62
Takata, 2007           -1.67 1.12
Devitt, 2011            0.66 0.43
Pouryazdanparast, 2012  0.62 0.65
Turri, 2012            -0.04 0.46


                         HR        95%-CI %W(fixed) %W(random)
Demunter, 2001         1.14  [0.47; 2.76]     23.91      23.91
Kumar, 2003            1.26  [0.38; 4.22]     12.76      12.76
Takata, 2007           0.19  [0.02; 1.70]      3.83       3.83
Devitt, 2011           1.94  [0.83; 4.53]     25.77      25.77
Pouryazdanparast, 2012 1.86  [0.52; 6.71]     11.30      11.30
Turri, 2012            0.96  [0.39; 2.39]     22.43      22.43

Number of studies combined: k=6

                       HR        95%-CI    z  p.value
Fixed effect model   1.26  [0.82; 1.94] 1.05   0.2935
Random effects model 1.26  [0.82; 1.94] 1.05   0.2935

Quantifying heterogeneity:
tau^2 < 0.0001; H = 1 [1; 1.9]; I^2 = 0% [0%; 72.4%]

Test of heterogeneity:
   Q d.f.  p.value
 4.6    5   0.4667

Details on meta-analytical method:
- Inverse variance method
- DerSimonian-Laird estimator for tau^2
```

## Forest plot



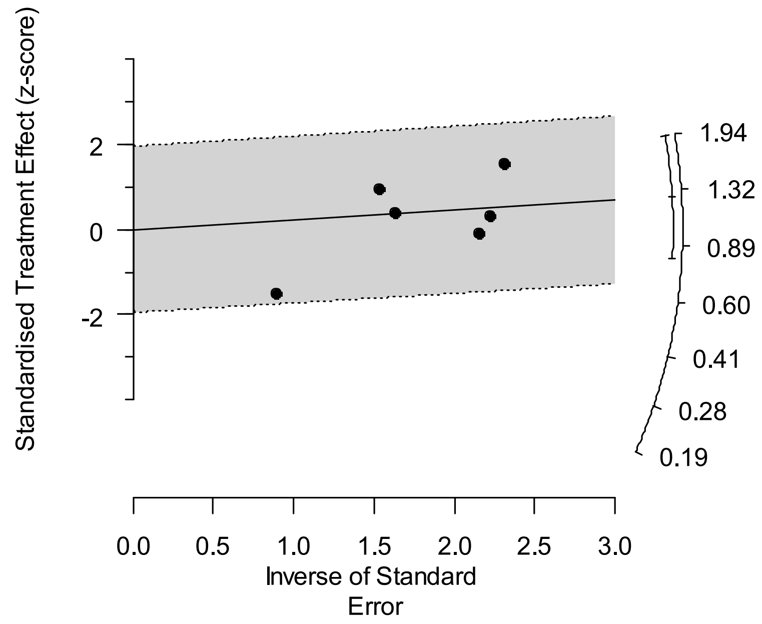

## Egger's test

```
        Linear regression test of funnel plot asymmetry

data:  nrasdfs.meta
t = -1.6018, df = 4, p-value = 0.1844
alternative hypothesis: asymmetry in funnel plot
sample estimates:
     bias   se.bias     slope
-2.035937  1.270995  1.286424
```

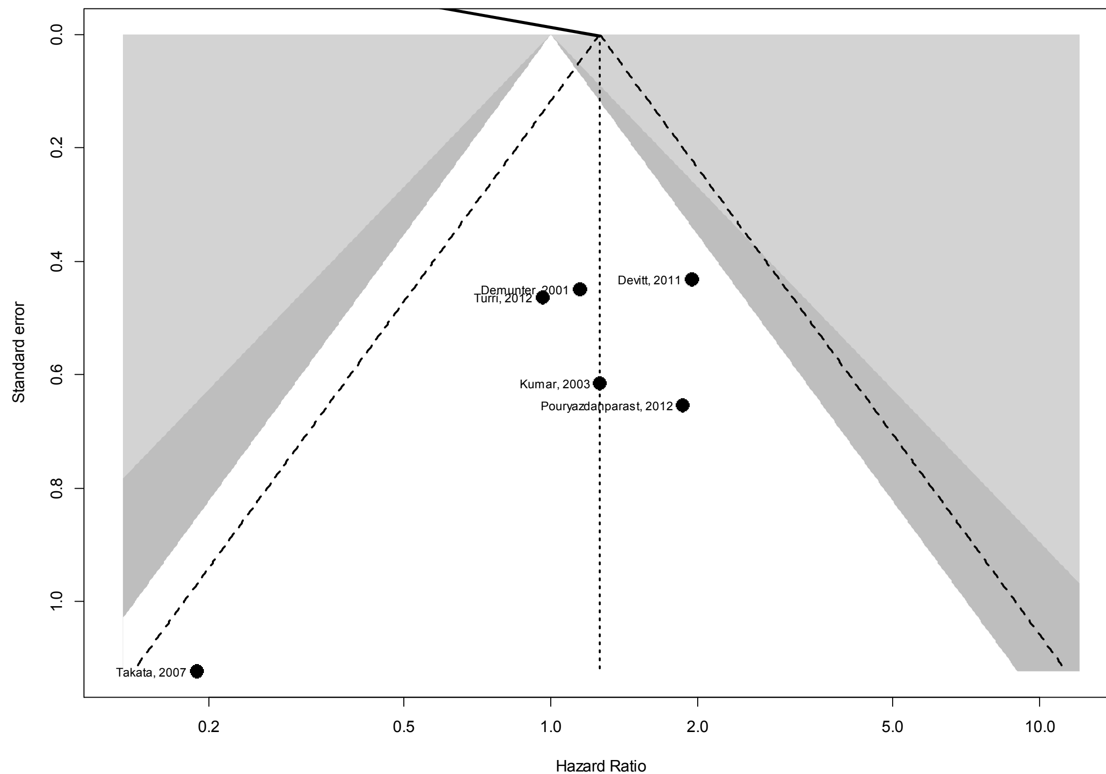
## Peters test

```
Regression Test for Funnel Plot Asymmetry

model:     mixed-effects meta-regression model
predictor: inverse of the total sample size

z = 0.7323, p = 0.4640
```

**Radial plot**



## Funnel plot




## Trim and Fill analysis

| | HR | 95%-CI | %W(fixed) | %W(random) |
|---|---|---|---|---|
| Devitt, 2011 | 1.9400 | [0.8304; 4.5322] | 25.77 | 25.77 |
| Demunter, 2001 | 1.1444 | [0.4743; 2.7613] | 23.91 | 23.91 |
| Pouryazdanparast, 2012 | 1.8643 | [0.5178; 6.7122] | 11.30 | 11.30 |
| Turri, 2012 | 0.9635 | [0.3880; 2.3922] | 22.43 | 22.43 |
| Takata, 2007 | 0.1883 | [0.0209; 1.7005] | 3.83 | 3.83 |
| Kumar, 2003 | 1.2626 | [0.3780; 4.2172] | 12.76 | 12.76 |

Number of studies combined: k=6 (with 0 added studies)

| | HR | 95%-CI | z | p.value |
|---|---|---|---|---|
| Fixed effect model | 1.2597 | [0.8189; 1.9378] | 1.0505 | 0.2935 |
| Random effects model | 1.2597 | [0.8189; 1.9378] | 1.0505 | 0.2935 |

Quantifying heterogeneity:

```
tau^2 < 0.0001; H = 1 [1; 1.9]; I^2 = 0% [0%; 72.4%]

Test of heterogeneity:
   Q d.f.  p.value
 4.6    5   0.4667

Details on meta-analytical method:
- Inverse variance method
- DerSimonian-Laird estimator for tau^2
- Trim-and-fill method to adjust for funnel plot asymmetry
```

## NRAS ONE STAGE COX IPD ANALYSIS

## DFS NRAS

```
Call:

  n= 111, number of events= 85
   (611 observations deleted due to missingness)

          coef exp(coef) se(coef)      z Pr(>|z|)
NRASwt -0.0198    0.9804   0.3243 -0.061    0.951

       exp(coef) exp(-coef) lower .95 upper .95
NRASwt    0.9804       1.02    0.5192     1.851

Concordance= 0.497  (se = 0.042 )
Rsquare= 0   (max possible= 0.984 )
Likelihood ratio test= 0  on 1 df,   p=0.9514
Wald test            = 0  on 1 df,   p=0.9513
Score (logrank) test = 0  on 1 df,   p=0.9513
```

## OS NRAS

```
Call:

  n= 649, number of events= 319
   (73 observations deleted due to missingness)

           coef exp(coef) se(coef)      z Pr(>|z|)
NRASwt -0.02852   0.97188  0.15679 -0.182    0.856

       exp(coef) exp(-coef) lower .95 upper .95
NRASwt    0.9719      1.029    0.7148     1.322

Concordance= 0.506  (se = 0.016 )
Rsquare= 0   (max possible= 0.983 )
Likelihood ratio test= 0.03  on 1 df,   p=0.856
Wald test            = 0.03  on 1 df,   p=0.8556
Score (logrank) test = 0.03  on 1 df,   p=0.8556
```

## MSS NRAS

```
Call:

  n= 146, number of events= 65
   (576 observations deleted due to missingness)

         coef exp(coef) se(coef)     z Pr(>|z|)
NRASwt 0.3356    1.3988   0.3261 1.029    0.303

       exp(coef) exp(-coef) lower .95 upper .95
NRASwt     1.399     0.7149    0.7382     2.651

Concordance= 0.536  (se = 0.06 )
Rsquare= 0.008   (max possible= 0.93 )
Likelihood ratio test= 1.13  on 1 df,   p=0.2884
Wald test            = 1.06  on 1 df,   p=0.3033
Score (logrank) test = 1.07  on 1 df,   p=0.3012
```

### *Summary of the Results*

Below is a summary of the results from the above analysis. The statistically significant results are shaded grey. HRs greater than 1 are suggestive of increased risk of death for BRAF mutated tumours. We did find an increased risk of death by 33% (p=0.054) in the random-effects and 27% in the fixed-effects (p=0.0058) analysis for BRAF mutated tumours compared to their wild-type counterparts. We found statistical significance for BRAF and MSS tumours only in a fixed-effects analysis. When combining OS and studies with MSS with did find a clear statistical significance for both random and fixed-effects models. No statistical significance was found for BRAF and DFS and none was found for any outcome for NRAS. Although no statistical significance was reached for DFS in the IPD data, interestingly enough the risk of metastasis seemed to be less for BRAF mutated tumours.

The statistical significance to OS for BRAF mutated tumours seems to relate to the metastatic tumours and stage IV melanoma cases and not to all cases. The risk of death when the time starts from the time of metastatic diagnosis is 44% for BRAF mutated cases (p=0.0298). The significance becomes less than 0.1 when we include also MSS studies. The statistical significance we see when we include all stages of melanoma is probably due to stage IV patients and so is for the analysis on metastatic tissue. The analysis on primary tissue does give also statistical results with the risk being higher for BRAF mutated tumours. However, this is due to the fixed-effects analysis. Once the heterogeneity increases, as we see by the OS+MSS analysis for this group, the random-effects analysis is not significant any more. The most heterogeneity seems to exist for the group that used both primary and metastatic tumour with no differentiation.

**Table 21: Summary of meta-analysis results for BRAF and NRAS.**

| | Number of studies / subjects | Model | HR | CI - lower 95% | CI - upper 95% | p-value |
|---|---|---|---|---|---|---|
| *BRAF* | | | | | | |
| ***Two-stage meta-analysis*** | | | | | | |
| **BRAF OS** | 10 | random | 1.33 | 0.99 | 1.77 | 0.0544 |
| | | fixed | 1.27 | 1.07 | 1.51 | 0.0058 |
| **BFAF MSS** | 6 | random | 1.61 | 0.90 | 2.88 | 0.1056 |
| | | fixed | 1.55 | 1.11 | 2.16 | 0.0101 |
| **BRAF OS+MSS** | 13 | random | 1.30 | 1.01 | 1.68 | 0.0448 |
| | | fixed | 1.28 | 1.09 | 1.49 | 0.0021 |
| **BRAF DFS** | 10 | random | 1.11 | 0.81 | 1.52 | 0.5213 |
| | | fixed | 1.07 | 0.86 | 1.34 | 0.5458 |
| | | | | | | |
| ***One-stage IPD meta-analysis*** | | | | | | |
| **BRAF OS** | 683 | stratified | 1.36 | 1.07 | 1.72 | 0.0119 |
| **BFAF MSS** | 147 | stratified | 1.21 | 0.61 | 2.37 | 0.584 |
| **BRAF DFS** | 146 | stratified | 0.73 | 0.45 | 1.20 | 0.214 |
| | | | | | | |
| **BRAF OS for stage IV** | 183 | stratified | 1.24 | 0.87 | 1.77 | 0.226 |
| | | non-stratified | 1.53 | 1.09 | 2.16 | 0.014 |
| | | | | | | |
| | | | | | | |
| ***Special Groups*** | | | | | | |
| **BRAF OS from time of metastasis** | 2 | fixed | 1.44 | 1.04 | 1.99 | 0.0298 |
| | | random | 1.44 | 1.04 | 1.99 | 0.0298 |
| **BRAF OS+MSS from time of metastasis** | 3 | random | 1.44 | 1.11 | 1.87 | 0.0061 |
| | | fixed | 1.44 | 1.11 | 1.87 | 0.0061 |
| | | | | | | |
| | | | | | | |

| | | | | | | |
|---|---|---|---|---|---|---|
| **BRAF OS from time of primary diagnosis** | 8 | random | 1.30 | 0.88 | 1.93 | 0.1948 |
| | | fixed | 1.21 | 0.99 | 1.48 | 0.0564 |
| **BRAF OS+MSS from time of primary diagnosis** | 10 | random | 1.23 | 0.84 | 1.80 | 0.2971 |
| | | fixed | 1.19 | 0.98 | 1.45 | 0.0744 |
| | | | | | | |
| | | | | | | |
| **BRAF OS when analysis is done on primary melanoma tissue** | 5 | random | 1.34 | 1.05 | 1.71 | 0.0207 |
| | | fixed | 1.34 | 1.05 | 1.71 | 0.0207 |
| **BRAF OS+MSS when analysis is done on primary melanoma tissue** | 6 | random | 1.22 | 0.89 | 1.68 | 0.2257 |
| | | fixed | 1.28 | 1.01 | 1.64 | 0.043 |
| | | | | | | |
| | | | | | | |
| **BRAF OS when analysis is done on metastatic melanoma tissue** | 2 | random | 1.44 | 1.04 | 1.99 | 0.0298 |
| | | fixed | 1.44 | 1.04 | 1.99 | 0.0298 |
| **BRAF OS+MSS when analysis is done on metastatic melanoma tissue** | 2 | random | 1.44 | 1.04 | 1.99 | 0.0298 |
| | | fixed | 1.44 | 1.04 | 1.99 | 0.0298 |
| | | | | | | |
| | | | | | | |
| **BRAF OS in studies that include all stages of melanoma** | 7 | random | 1.49 | 1.04 | 2.15 | 0.031 |
| | | fixed | 1.47 | 1.16 | 1.85 | 0.0013 |
| **BRAF OS+MSS in studies that include all stages of melanoma** | 9 | random | 1.38 | 0.94 | 2.03 | 0.0986 |
| | | fixed | 1.42 | 1.13 | 1.79 | 0.0025 |
| | | | | | | |

| *NRAS* | | | | | | |
|---|---|---|---|---|---|---|
| ***Two-stage random-effects meta-analysis*** | | | | | | |
| **NRAS OS** | 8 | random<br>fixed | 1.16<br>1.15 | 0.79<br>0.93 | 1.71<br>1.42 | 0.4415<br>0.1962 |
| **NRAS MSS** | 4 | random<br>fixed | 1.43<br>1.40 | 0.64<br>0.80 | 3.20<br>2.47 | 0.3833<br>0.2426 |
| **NRAS OS+MSS** | 10 | random<br>fixed | 1.17<br>1.15 | 0.81<br>0.93 | 1.69<br>1.41 | 0.3975<br>0.1892 |
| **NRAS DFS** | 6 | random<br>fixed | 1.26<br>1.26 | 0.82<br>0.82 | 1.94<br>1.94 | 0.2935<br>0.2935 |
| | | | | | | |
| ***One-stage random-effects meta-analysis*** | | | | | | |
| **NRAS OS** | 649 | stratified | 1.03 | 0.76 | 1.40 | 0.856 |
| **NRAS MSS** | 146 | stratified | 0.71 | 0.38 | 1.35 | 0.303 |
| **NRAS DFS** | 111 | stratified | 1.02 | 0.54 | 1.93 | 0.951 |

## *Discussion*

Melanoma is an aggressive cancer with an increasing incidence (Thompson et al., 2009). In contrast to other skin cancers, melanoma affects a younger population and has a stronger tendency to metastasize with a consequently extremely poor overall survival. Lately, oncogenes have been found in melanoma, as well as in other cancers which may play a role on either oncogenesis or progression to metastases. Melanocytes acquire stepwise abnormalities of oncogenes (Miller and Mihm, 2006) and there is evidence that the frequency of these molecular transformations differ between the various subtypes of melanoma, supporting the hypothesis that melanoma is a molecular heterogeneous disease (Curtin et al., 2005). In our systematic review we searched over the available literature and tried to identify the prognostic value of two of those genes in melanoma: BRAF and NRAS. The corresponding proteins of those genes are involved in sending signals inside cells which regulate cell growth.

BRAF mutations are a lot more common in cutaneous malignant melanoma than other oncogenic mutations, such as NRAS, p16INK4a, and p53. Mutations in the B-RAF gene are mainly localized in the kinase activation domain with the majority involving the substitution of valine by acidic or basic residues at codon 600 (Brose et al., 2002; Davies et al., 2002). NRAS and BRAF mutations rarely co-exist, suggesting that they may possess important overlapping oncogenic activities (Brose et al., 2002; Omholt et al., 2002; Kumar et al., 2003b; Omholt et al., 2003; Houben et al., 2004; Akslen et al., 2005; Curtin et al., 2005). Similar results (i.e., that RAS and BRAF mutations never seem to coexist in the same lesion) have also been reported in other tumour types, including colorectal, ovarian, and papillary thyroid carcinomas (Cohen et al., 2004).

Activating mutations in the BRAF oncogene have been reported in 33% to 47% (Houben et al., 2004; Curtin et al., 2005; Liu et al., 2007; Thomas et al., 2007;

Viros et al., 2008) of primary melanomas and 41% to 55% of metastatic melanomas (Gorden et al., 2003; Kumar et al., 2003a; Chang et al., 2004; Houben et al., 2004; Kirschner et al., 2005; Ugurel et al., 2007). In our systematic review, activating mutations in the BRAF oncogene have been reported between 20% and 59% for primary melanomas (Omholt et al., 2003; Casula et al., 2004; Shinozaki et al., 2004; Akslen et al., 2005; Edlundh-Rose et al., 2006; Brown et al., 2012) and between 48% and 69% for metastatic melanoma (Ugurel et al., 2007; Long et al., 2011; Brown et al., 2012). On the contrary, NRAS mutations were found only between 5% and 29% (Demunter et al., 2001; Omholt et al., 2002; Houben et al., 2004; Akslen et al., 2005; Edlundh-Rose et al., 2006; Turri-Zanoni et al., 2012). The differences may represent variations in the sample composition and characteristics, as well as differences in the sensitivity of the method used for the detection of the mutations. It has also been largely attributed to the differential source of tumour DNA analysed, e.g., fixed tumour tissues or in vitro propagated melanoma cells. In our meta-analysis we included only studies that have analysed tissue specimens, although some of them may have analysed cell lines in parallel as well. However, there were other differences between the studies in either their specimens, DNA extraction or population composition that may affect the percentage of BRAF mutated samples. Menzies et al., 2012 have reported that failure to detect V600K BRAF mutations may prevent up to 30% of the primary and 15% of the metastatic melanoma population to gaining access to trials with BRAF inhibitors (Menzies et al., 2012). Also, referral bias depending on hierarchy of the centre in the local health system can be another reason. However, when combined, BRAF and NRAS genes can be mutated in up to 55% of the primary tumours, supporting a significant role in melanoma development (Akslen et al., 2005).

There were many differencies between the studies in our analysis. There were differences in the fixation of the tissue. Some reasearchers used paraffin embedded tissue samples (van Elsas et al., 1996; Omholt et al., 2003; Deichmann et al., 2004; Shinozaki et al., 2004; Janssen et al., 2008; Broekaert et

al., 2010; Caramuta et al., 2010; Devitt, 2011; Long et al., 2011), whereas others used fresh frozen (Ugurel et al., 2007) or both (Edlundh-Rose et al., 2006). In our analysis there was not any statistical significant difference in our results when accounting for the fixation type.

Not all researchers screened for mutations in the same exons. More than 80 somatic mutations in exon 15 of the BRAF gene have been identified in melanoma (Arkenau et al., 2011). All researchers who screened for BRAF have looked into exon 15, but only 4 researchers looked into exon 11 as well (Omholt et al., 2003; Shinozaki et al., 2004; Edlundh-Rose et al., 2006; Ugurel et al., 2007). The V600E (thymine to adenine) mutation is the most prevalent, followed by the V600K mutation (valine to lysine). According to Menzies et al. the V600E have a higher proportion on the extremities, whereas V600K is more often found in the head and neck region (Menzies et al., 2012). Turri-Zanoni et al. (2012) support this as they found that all primary sinonasal mucosal melanomas lack the BRAF V600E mutation. (Turri-Zanoni et al., 2012) Also, all researchers screened for NRAS at codon 61 but different exons. Devitt (Devitt, 2011) screened in exon 3 of the NRAS gene, van Elsas et al., Demunter et al. and Ugurel et al. and (van Elsas et al., 1996; Demunter et al., 2001; Ugurel et al., 2007) in exons 1 and 2, Edlundh-Rose et al., Omholt et al. and Caramuta et al. in 2 (Omholt et al., 2002; Omholt et al., 2003; Edlundh-Rose et al., 2006; Caramuta et al., 2010) and Broekaert et al. does not mention (Omholt et al., 2003; Broekaert et al., 2010). As pointed out by Omholt et al. NRAS codon 61 mutations are the most common RAS alterations in human melanoma. NRAS (22%) and KIT (12.5%) are also present in sinonasal mucosal melanomas (Turri-Zanoni et al., 2012). Mutations may be present in other parts of those genes as well, rather than those screened, making the wild-type a heterogeneous group. Demunter et al., have discovered a new NRAS mutation in codon 18 which seems to improve survival as these tumours carrying this mutation seem to lack metastasizing ability and be significantly thinner than melanoma tumours carrying mutations in codons 12 and 61 (Demunter et al., 2001). This may affect up to 15% of melanoma tumours.

However, no other researcher has confirmed their findings. Even overexpression of wild-type BRAF due to gene amplification could be involved in growth of malignant melanoma cell lines (Tanami et al., 2004). Moreover, alternative candidate genes in the MAPK pathway, which could possibly affect survival include MEK1 and 2, acting immediately downstream of BRAF (Emery et al., 2009), and have not been analysed in the current series. However, there wasn't any statistical significant difference in our results by grouping according to the above factor.

Our results show that mutations in BRAF and NRAS occurring in cutaneous melanoma are associated with different pathological characteristics and possibly clinical behaviour. BRAF mutation seems to be associated with younger age (Broekaert et al., 2010; Devitt, 2011; Long et al., 2011; Jakob et al., 2012; Menzies et al., 2012). This is also supported by other studies (Maldonado et al., 2003; Viros et al., 2008; Thomas et al., 2010). Si et al. have also found patients with NRAS mutations to be older that their wild-type counterparts. (Si et al., 2012)

The incidence of BRAF mutation did not correlate with Breslow thickness (Shinozaki et al., 2004; Long et al., 2011) or ulceration (Devitt, 2011; Long et al., 2011). Some researchers found a greater tendency for ulceration in BRAF mutated tumours than in those with NRAS mutations, which for Akslen was statistically significant (73% vs 58%, $p=0.039$) (Akslen et al., 2005; Edlundh-Rose et al., 2006). Si et al. found a significantly higher ulceration rate in patients with BRAF (61.1%, $p<0.01$) and NRAS mutations (64%, $p=0.04$) than that in patients without BRAF (40.9%) or NRAS mutations (40.7%) (Si et al., 2012). On the other hand, NRAS mutations were associated with a higher Clark level of invasion than BRAF mutations (Edlundh-Rose et al., 2006) or higher Breslow thickness (Devitt, 2011). Devitt has failed to find an association between NRAS and sun exposure, however van Elsas et al., (1996) did.

Shinozaki et al. (2004) observed a trend for greater incident of BRAF mutation in female patients, although this was not statistically significant. Jacob et al., observed NRAS mutations to be less common in Hispanic patients (8.8%) compared to other Caucasian patients (21.1%) (Jakob et al., 2012). They have also noticed that Asian and black patients had an increase wild-type frequency to Caucasians.

It could also be that the high frequency of BRAF mutations may vary between studies because of differences in the distribution of histopathologic subtypes (Edlundh-Rose et al., 2006). BRAF was correlated with the histopathologic subtype of superficial spreading (Devitt, 2011; Long et al., 2011; Menzies et al., 2012) whereas the nodular melanoma was commoner for NRAS mutations (Devitt, 2011). There are reports that BRAF is less frequent in acral lentiginous and lentigo maligna melanoma (Curtin et al., 2005; Si et al., 2012). On the contrary NRAS mutations were specifically associated with nodular melanoma and to a lesser extent with lentigo malignant melanoma (van Elsas et al., 1996). Relations may be different due to the different composition of races. Acral melanomas account for only 1–7% of all cutaneous melanomas in Caucasians, whereas the percentage is significantly higher (up to 42%) in Asian population (Byrd-Miles et al., 2007; Chi et al., 2011).

Some authors included only certain types of melanoma, like nodular (Akslen et al., 2005). Very few included mucosal melanomas (Si et al., 2012) whereas other analysed only ocular (Janssen et al., 2008) or only sinonasal tumours (Turri-Zanoni et al., 2012). Takata, et al. analysed spitz naevus as well as melanoma cases (Takata et al., 2007). Spitz naevus has been defined as a special variant of benign melanocytic naevus that shares many histopathological features with malignant melanoma and some of them give later rise to metastasis. They conclude that melanoma and Spitz naevus are distinct tumours. Casula, et al. (2004) confirm that they also detect BRAF mutations in many histologically

different nevi and argue that although a crucial step, mutation of this gene alone is insufficient for the development of melanoma.

It also seems that BRAF is associated with fewer markers of chronic sun damage (CSD) in surrounding skin (Maldonado et al., 2003; Edwards et al., 2004; Devitt, 2011; Long et al., 2011; Si et al., 2012) and higher total body nevus counts (Edlundh-Rose et al., 2006; Long et al., 2011). None of the BRAF mutations are of CC>TT or C>T substitutions, which are associate with pyrimidine dimer formation following exposure to UV light and are common in UV-induced carcinogenesis (Davies et al., 2009) supporting the evidence that BRAF may not be UV related mutation. Menzies, et al. (2012) support that this may not be true for the BRAF V600K mutation. Not all authors though found a correlation of BRAF with sun exposure (Brown et al., 2012; Menzies et al., 2012). However, Menzies, et al. found that V600K BRAF mutant melanomas are associated with high levels chronic sun damage, whereas V600E are associated with little or no sun damage. Interestingly, they also found that the frequency of non-V600E genotypes increased with increasing age-decade. On the other hand, UV light has been proven to induce activation of the NRAS gene (Van der Lubbe et al., 1988) possibly early during melanoma development (Omholt et al., 2002). The CAA(Gln)-AAA(Lys) mutation may arise from mispairing of adenosine with 8-oxo-deoxyguanosine and other mutations occur opposite dipyrimidine sequences.

Edlundh-Rose, et al. (2006) and Menzies, et al. (2012) found that BRAF mutations tended to be most frequent in melanomas on the trunk and lower extremities, sites associated with a pattern of intermittent sun exposure, and less frequent in head and neck. On the other hand, they found NRAS mutations were most common in tumours on the upper extremities with a more chronic sun exposure pattern. Van Elsas, et al. (1996) have also found increased NRAS mutated tumours in areas coming from the face and head compared to other areas. Broedaert, et al. (2010) found a similar association for BRAF and the trunk but not for acral location. These results are in agreement with those of other

researchers, not included in our current analysis (Jiveskog et al., 1998; Maldonado et al., 2003; Curtin et al., 2005). Si, et al. (2012) found that the highest frequency (46.7%) of BRAF mutation was detected in mucosal melanomas located in sinonasal mucosa. Other researchers did not share the same findings for anatomical sites (Shinozaki et al., 2004).

There were also some researchers who failed to find any correlation of BRAF or NRAS mutations with gender (Si et al., 2012), age at diagnosis (Brown et al., 2012; Si et al., 2012), clinical stage at diagnosis (Kumar et al., 2003a; Casula et al., 2004; Si et al., 2012) (BRAF), histogenetic type (Si et al., 2012) (NRAS), level of tumour invasion according to Clark (Casula et al., 2004), tumour thickness (Casula et al., 2004; Akslen et al., 2005; Brown et al., 2012; Jakob et al., 2012; Si et al., 2012), ulceration (Brown et al., 2012; Jakob et al., 2012; Si et al., 2012) (BRAF), and site of first recurrence (Omholt et al., 2003). Many researchers failed to find statistical significance for know prognostic factors of melanoma, like thickness, age or stage. We believe that is possible due to small numbers or selection bias. We certainly did find significance for these factors in our IPD meta-analysis.

Jacob, et al. (2012) found a non-significant trend of NRAS and wild-type patients to have less frequently elevated LDH than BRAF patients. They have also found a higher rate of CNS involvement among the BRAF (24.4%, p=0.01) and NRAS patients (23.1%, p=0.056) compared to wild-type patients (12.4%). However, the rate reversed with regard to the rate of lung metastases with BRAF (55.2%) and NRAS (56.7%) patients to be lower to the wild-type patients (66.9%) at stage IV presentation. Also, Si, et al. (2012) did find that patients with NRAS mutations were more likely to be at disease stage III than the wild-type patients. Brown et al., (2012) have found BRAF mutations to be associated with increase pigmentation in primary melanomas, as have other researchers. (Liu et al., 2007; Viros et al., 2008)

Some authors found more mutations in metastatic melanomas (Shinozaki et al., 2004), whereas others did not support this (Omholt et al., 2003; Edlundh-Rose et al., 2006). Akslen, et al. (2005) found only two cases with a mutation (18%) in paired metastases, in patients without BRAF mutations in their primary tumour. In our analysis we did find more BRAF mutations in later stages of melanoma and that was statistically significant. Omholt, et al. (2002) suggests that with regard to NRAS, codon 61 mutations first appeared in the primary tumours rather than in the metastases, indicating that they are early events in melanoma tumour development. Additionally, in none of the patients with primary tumours that were wild-type for NRAS codon 61 did mutations arise in the metastases, indicating that those mutations may not be a critical event for metastasis initiation. Moreover, Edlundh-Rose, et al. (2006) found that tumours with BRAF mutations showed a significantly more frequent moderate to pronounced infiltration of lymphocytes. Broekaert, et al. (2010) and Shinozaki, et al. (2004) also found that they metastasized more frequently to regional lymph nodes. Akslen, et al. (2005) has found that tumours with BRAF mutation are more likely to give metastases than BRAF mutation-negative.

The locality of the samples was also different. Van Elsas, et al. (1996) found that OS for NRAS, was better in Europe than in Australia. Similar findings are reported by other researchers as well.

Based on all the above some authors suggest that using a combination of morphometric features, BRAF mutation status could be predicted with 82% accuracy (Viros et al., 2008). Other researchers have created a "molecular disease model" for melanoma that classifies individual tumours into molecular subtypes with proposed treatment guidelines for each based on the different molecular characteristics of different histopathologic types of melanoma. (Vidwans et al., 2011)

With regard to survival, many authors did not find any statistically significant association between the presence of mutation in BRAF in a primary melanoma and disease free survival (DFS) (Casula et al., 2004; Deichmann et al., 2004; Shinozaki et al., 2004; Akslen et al., 2005; Broekaert et al., 2010; Long et al., 2011; Menzies et al., 2012) or overall survival (OS) (Casula et al., 2004; Akslen et al., 2005; Edlundh-Rose et al., 2006; Janssen et al., 2008; Broekaert et al., 2010; Devitt, 2011; Long et al., 2011; Brown et al., 2012; Jakob et al., 2012; Pouryazdanparast et al., 2012). However, it was associated with a worse outcome thereafter, i.e for metastatic melanoma (Ugurel et al., 2007; Long et al., 2011). This has previously been reported in colorectal cancer (Roth et al., 2010). If a BRAF mutation has no effect on the DFS yet impacts survival after diagnosis of distant metastases, it suggests that the presence of a BRAF mutation provides an essential genetic background for more rapid evolution of the metastatic phenotype once early metastatic events occur (Long et al., 2011). However, Brown, et al. (2012) has found a statistically significant shorter DFS (HR=2.62, p=0.02) and Si, et al. (2012) a statistically significant shorter OS (33 vs 53 months, p=0.005) for patients with the BRAF mutation. Menzies, et al. (2012) found a significantly shorter DFS for patients with the BRAF V600K mutation. However, as these patients are older, their result needs to be interpreted with caution as these patients may die from other causes before they develop metastatic disease. Kumar, et al. (2003a) has found a longer DFS in metastatic patients with a BRAF mutation, although the finding was not significant. They hypothesize that the effect of BRAF mutations can potentially override the effect of widespread genomic destabilization and therefore prolong the disease progression period. However, this mutation induces also resistance to cisplatin and radiotherapy. We should also mention here a novel research by Shinozaki, et al. (Shinozaki et al., 2007). They tried to identify BRAF V600E mutation on exon 15 as circulating DNA in the serum of melanoma patients with the aim to see if there is any significant correlation to disease outcome and whether it could be useful in patient follow-up for monitoring disease progression. They did find an association with poor outcomes and significantly lower overall survival.

In our analysis we did find statistically significant worse OS for melanoma patients with the BRAF mutation. We did not find any statistical significant difference for the DFS though. In the IPD analysis the DFS was longer for BRAF melanoma patients but that was not statistically significant. However, it agrees with what Kumar, et al. (2003a) has found. Our analysis was the first to include an IPD meta-analysis of almost 700 cases for both BRAF and NRAS genes in melanoma. With regard to the extraction of data, we used a digitizer to split the K-M curve in 20 or more pieces before extracting. To the best of our knowledge, although the technique is reported, no-one else has tried it in a meta-analysis. That gave us significant accuracy of the estimated result, although due to the method the variance still has to stay large. We did find significant HR for the BRAF gene compared to wild-type patients. This remained even in the random effects analysis despite the heterogeneity. The HR seems to be significant only in metastatic melanoma cases in both the aggregate and individual patient data analysis. This has proven difficult to prove for MSS due to the smaller numbers. Our results must be interpreted with care. We did notice an increase in BRAF mutated tumours with later stages of melanoma. That could be true or a selection bias, due to more tissue for analysis and therefore more positive results. Also, although we made sure to select patients that have not received any BRAF inhibitors, this may not have been shared by the authors of the studies we used. In any case, these results reach high statistical significance to be ignored.

On the contrary, NRAS patients showed a trend towards a favourable survival compared to wild-type patients in metastatic melanoma but this did not reach statistical significance (van Elsas et al., 1996; Ugurel et al., 2007). If that is true, then NRAS, in contrast to findings in other human cancers and in contrast to its oncogene function, may be a favourable prognostic factor in human melanoma. However, other researchers found that patients with NRAS mutations experienced shorter MSS and DFS although once patients relapsed the mutational state did not influence the time to death (Devitt, 2011; Jakob et al.,

2012; Pouryazdanparast et al., 2012). This difference was attributable to shorter relapse-free survival after initial treatment of locoregional disease among the NRAS patients. Jakob, et al. (2012) had similar findings for stage IV patients, even those who underwent molecular testing within 6 months of their stage IV diagnosis, so as to avoid enrichment with long survival patients and stage IV disease. However, they have no explanation for the shorter survival from stage IV for the NRAS-mutant patients, as the presence of the mutation did not correlate with other established stage IV prognostic factors, such as elevated LDH or advanced M1 category. Si, et al. (2012) found that patients with NRAS mutations had a worse survival (33.0 months) than patients with wild-type tumours (48.0 months; p = 0.031). Akslen, et al. (2005) and Turri-Zanoni, et al. (2012) and found no difference in survival in patients with NRAS mutations, although Akslen analysed only nodular melanomas and Turri-Zanoni only sinonasal melanomas. Our meta-analysis was the first to examine the NRAS gene in melanoma. In our analysis we did not find any statistical significant result with regard to the prognostic value of the NRAS gene in melanoma patients in either the two-stage meta-analysis or one-stage IPD meta-analysis. We hypothesize that this will not change with new research.

Some researchers have also shown that when patients with metastatic melanoma and mutated BRAF oncogene are treated with a mutant-selective BRAF inhibitor (Bollag et al., 2010; Flaherty et al., 2010) they can achieve a better prognosis (Long et al., 2011; Jakob et al., 2012; Menzies et al., 2012; Si et al., 2012). The inhibitors now in trials are vemurafenib, dabrafenib and dacarbazine and are improving the management of patients with BRAF-mutant metastatic melanoma. Early phase trials of highly selective agents targeting BRAF and KIT show promising results (Carvajal et al., 2009, Flaherty et al., 2010, Hatzivassiliou et al., 2010). With regard to NRAS, van Elsas, et al. (1996) found that it was associated with favourable response to immunotherapy, using a-interferon plus interleukin-2. However, these results must also be viewed with

caution as they do not involve all the included patients and we do not know why the other patients were not enrolled in these trials.

Further study is needed into examination of PTEN, CCND1, and CDKN2A as aberrations of these genes are likely to interact with BRAF and NRAS to further drive clinical outcome.

One problem of many of the included studies is that the melanoma patients that were selected consisted almost exclusively of patients who had developed metastases and were therefore enriched for patients with a poor prognosis. This may result in skewed results in a survival analysis and may explain the inability of some of the researchers to show the significant influence of other well documented prognostic factors, such as ulceration, gender or histopathologic type (Jakob et al., 2012). On the other hand, in the same retrospective articles, the analysis of survival may be biased by the inclusion of patients who survived with stage IV disease for many years before the implementation of molecular testing. Very few authors included all stages of melanoma in their research (Brown et al., 2012).

## *Conclusion*

We found that the BRAF mutation affects negatively the overall survival in melanoma patients by increasing the risk of death by 30 - 36% (depending on the method) compared to wild-type cases and this is statistically significant (p-value=0.00119 – 0.0544, depending on the method). However, this seems to be true mainly for metastatic cases where the increase of risk is 44% (p-value=0.0298) and 53% for stage IV and not for all the stages of melanoma. There is also a statistically significant increase of the BRAF mutation in higher stages of melanoma but we cannot say whether this is a true effect or a selection of cases bias. We did not find any statistically significant result for DFS, although

interstingly enough, the BRAF mutation may be beneficial to survival in those cases (from IPD one-stage random-effects analysis). More IPD data are needed to find statistically significant results for DFS and MSS for BRAF mutated cases. We found no statistically significant effect for any outcome measure with regard the NRAS mutation. Further research is needed and we hope that this study will be the basis for better designed studies on this topic.

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## *Appendix*

## The code for the above analysis for analysis in R

```
###### The following is for package meta ########################
################################################################


path<-"Z:\\DATA\\"

sink("Melanoma_Meta.txt", append=FALSE, split=TRUE)

library("metafor")

library("meta")


# ###BRAF DFS ###

# melanoma.meta<-"Final_Aggregate.csv"

# brafnras<-read.csv(paste(path,melanoma.meta,sep=""),header=TRUE)

# brafdfs<-subset(brafnras, brafnras$BRAF=="1" & brafnras$include=="1" &
brafnras$DFS_Inc=="1")

melanoma.metadfs<-"Final_Aggregate_braf_dfs.csv"

brafdfs<-read.csv(paste(path,melanoma.metadfs,sep=""),header=TRUE)

brafdfs.meta<- metagen(TE=brafdfs$ln_hr_dfs_braf,
seTE=brafdfs$se_ln_hr_dfs_braf, studlab=paste(brafdfs$author, brafdfs$Year,
sep=", "), sm="HR", level=0.95, level.comb=0.95, comb.fixed=TRUE,
comb.random=TRUE, hakn=FALSE, method.tau="DL", tau.preset=NULL, TE.tau=NULL,
method.bias="linreg", n.e=brafdfs$braf_n, n.c=brafdfs$braf_n_wt, title="HR_DFS
for BRAF", complab="", outclab="", label.e="BRAF", label.c="WT", label.left="",
label.right="", warn=TRUE)
```

```
print(brafdfs.meta, sortvar=brafdfs$Year, level=brafdfs.meta$level,
level.comb=brafdfs.meta$level.comb, comb.fixed=brafdfs.meta$comb.fixed,
comb.random=brafdfs.meta$comb.random, details=TRUE, ma=TRUE, digits=2)

win.metafile(filename = "Forest_brafdfs.wmf", width = 10, height = 4.5,
pointsize = 12, restoreConsole = FALSE)
forest(brafdfs.meta, smlab="HR DFS BRAF", sortvar=brafdfs$Year)
dev.off()

# metabias(brafdfs.meta, method.bias="rank", plotit=TRUE, correct=TRUE,
k.min=3)
metabias(brafdfs.meta, method.bias="linreg", plotit=TRUE, correct=FALSE,
k.min=3)
# metabias(brafdfs.meta, method.bias="mm", plotit=TRUE, correct=FALSE, k.min=3)

# metabias(brafdfs.meta, method.bias="count", plotit=FALSE, correct=TRUE,
k.min=3)
# method.bias 'count' only defined for meta-analysis with binary outcome data
(function 'metabin')
# metabias(brafdfs.meta, method.bias="score", plotit=TRUE, correct=FALSE,
k.min=3)
# method.bias 'score' only defined for meta-analysis with binary outcome data
(function 'metabin')
# metabias(brafdfs.meta, method.bias="peters", plotit=FALSE, correct=FALSE,
k.min=3)
# method.bias 'peters' only defined for meta-analysis with binary outcome data
(function 'metabin')

metainf(brafdfs.meta, pooled="random", sortvar=brafdfs$Year,
level.comb=brafdfs.meta$level.comb)

metacum(brafdfs.meta, pooled="random", sortvar=brafdfs$Year,
level.comb=brafdfs.meta$level.comb)

win.metafile(filename = "Funnel_brafdfs.wmf", width = 12, height = 9, pointsize
= 12, restoreConsole = FALSE)
funnel(brafdfs.meta, comb.fixed=FALSE, comb.random=TRUE, level=0.95,
contour=c(0.9, 0.95, 0.99), col.contour=c("white", "grey", "lightgrey"), lwd=2,
cex=2, pch=16, studlab=TRUE, cex.studlab=0.75)
dev.off()
```

```
# win.metafile(filename = "Radial_brafdfs.wmf", width = 9, height = 5,
pointsize = 12, restoreConsole = FALSE)
# radial(brafdfs.meta, comb.fixed=TRUE, axes=TRUE, cex=1, pch=16, level=0.95)
# dev.off()

brafdfs.trim<-trimfill(brafdfs.meta)

print(brafdfs.trim)

win.metafile(filename = "Funnel_brafdfs.trim.wmf", width = 12, height = 9,
pointsize = 12, restoreConsole = FALSE)
funnel(brafdfs.trim, comb.fixed=FALSE, comb.random=TRUE, level=0.95,
contour=c(0.9, 0.95, 0.99), col.contour=c("white", "grey", "lightgrey"), lwd=2,
cex=2, pch=16, studlab=TRUE, cex.studlab=0.75)
dev.off()




# path<-"Z:\\DATA\\"

# library("metafor")

# melanoma.metadfs<-"Final_Aggregate_braf_dfs.csv"

# brafdfs<-read.csv(paste(path,melanoma.metadfs,sep=""),header=TRUE)

# brafdfs.meta<-rma.uni(yi=ln_hr_dfs_braf, sei=se_ln_hr_dfs_braf, data=brafdfs,
measure="GEN", slab=paste(brafdfs$author, brafdfs$Year, sep=", "), method="DL",
knha=FALSE, level=95)

# regtest.rma(brafdfs.meta, model="rma", predictor="ninv",
ni=brafdfs$braf_n_total)

# win.metafile(filename = "Radial_brafdfs_2.wmf", width = 6, height = 5,
pointsize = 12, restoreConsole = FALSE)
# radial(brafdfs.meta, transf=exp, xlab="Inverse of Standard Error",
zlab="Standardised Treatment Effect (z-score)")
# dev.off()
```

```
# ### BRAF OS ###

# library("metafor")

# library("meta")

# melanoma.meta<-"Final_Aggregate.csv"

# brafnras<-read.csv(paste(path,melanoma.meta,sep=""),header=TRUE)

# brafos<-subset(brafnras, brafnras$BRAF=="1" & brafnras$include=="1" &
brafnras$OS_Inc=="1")

melanoma.metaos<-"Final_Aggregate_braf_os.csv"

brafos<-read.csv(paste(path,melanoma.metaos,sep=""),header=TRUE)

brafos.meta<- metagen(TE=brafos$ln_hr_os_braf, seTE=brafos$se_ln_hr_os_braf,
studlab=paste(brafos$author, brafos$Year, sep=", "), sm="HR", level=0.95,
level.comb=0.95, comb.fixed=TRUE, comb.random=TRUE, hakn=FALSE,
method.tau="DL", tau.preset=NULL, TE.tau=NULL, method.bias="linreg",
n.e=brafos$braf_n, n.c=brafos$braf_n_wt, title="HR_OS for BRAF", complab="",
outclab="", label.e="BRAF", label.c="WT", label.left="", label.right="",
warn=TRUE)

print(brafos.meta, sortvar=brafos$Year, level=brafos.meta$level,
level.comb=brafos.meta$level.comb, comb.fixed=brafos.meta$comb.fixed,
comb.random=brafos.meta$comb.random, details=TRUE, ma=TRUE, digits=2)

win.metafile(filename = "Forest_brafos.wmf", width = 10, height = 4.5,
pointsize = 12, restoreConsole = FALSE)
forest(brafos.meta, smlab="HR OS BRAF", sortvar=brafos$Year)
dev.off()

# metabias(brafos.meta, method.bias="rank", plotit=TRUE, correct=TRUE, k.min=3)
metabias(brafos.meta, method.bias="linreg", plotit=TRUE, correct=FALSE,
k.min=3)
# metabias(brafos.meta, method.bias="mm", plotit=TRUE, correct=FALSE, k.min=3)
```

```
# metabias(brafos.meta, method.bias="count", plotit=FALSE, correct=TRUE,
k.min=3)
# method.bias 'count' only defined for meta-analysis with binary outcome data
(function 'metabin')
# metabias(brafos.meta, method.bias="score", plotit=TRUE, correct=FALSE,
k.min=3)
# method.bias 'score' only defined for meta-analysis with binary outcome data
(function 'metabin')
# metabias(brafos.meta, method.bias="peters", plotit=FALSE, correct=FALSE,
k.min=3)
# method.bias 'peters' only defined for meta-analysis with binary outcome data
(function 'metabin')

metainf(brafos.meta, pooled="random", sortvar=brafos$Year,
level.comb=brafos.meta$level.comb)

metacum(brafos.meta, pooled="random", sortvar=brafos$Year,
level.comb=brafos.meta$level.comb)

win.metafile(filename = "Funnel_brafos.wmf", width = 12, height = 9, pointsize
= 12, restoreConsole = FALSE)
funnel(brafos.meta, comb.fixed=FALSE, comb.random=TRUE, level=0.95,
contour=c(0.9, 0.95, 0.99), col.contour=c("white", "grey", "lightgrey"), lwd=2,
cex=2, pch=16, studlab=TRUE, cex.studlab=0.75)
dev.off()

# win.metafile(filename = "Radial_brafos.wmf", width = 9, height = 5, pointsize
= 12, restoreConsole = FALSE)
# radial(brafos.meta, comb.fixed=TRUE, axes=TRUE, cex=1, pch=16, level=0.95)
# dev.off()

brafos.trim<-trimfill(brafos.meta)

print(brafos.trim)

win.metafile(filename = "Funnel_brafos.trim.wmf", width = 12, height = 9,
pointsize = 12, restoreConsole = FALSE)
funnel(brafos.trim, comb.fixed=FALSE, comb.random=TRUE, level=0.95,
contour=c(0.9, 0.95, 0.99), col.contour=c("white", "grey", "lightgrey"), lwd=2,
cex=2, pch=16, studlab=TRUE, cex.studlab=0.75)
dev.off()
```

```
# path<-"Z:\\DATA\\"

# library("metafor")

# melanoma.metaos<-"Final_Aggregate_braf_os.csv"

# brafos<-read.csv(paste(path,melanoma.metaos,sep=""),header=TRUE)

# brafos.meta<-rma.uni(yi=ln_hr_os_braf, sei=se_ln_hr_os_braf, data=brafos,
measure="GEN", slab=paste(brafos$author, brafos$Year, sep=", "), method="DL",
knha=FALSE, level=95)

# regtest.rma(brafos.meta, model="rma", predictor="ninv",
ni=brafos$braf_n_total)

# win.metafile(filename = "Radial_brafos_2.wmf", width = 6, height = 5,
pointsize = 12, restoreConsole = FALSE)
# radial(brafos.meta, transf=exp, xlab="Inverse of Standard Error",
zlab="Standardised Treatment Effect (z-score)")
# dev.off()




# ### BRAF MSS ###

# library("metafor")

# library("meta")

# melanoma.meta<-"Final_Aggregate.csv"

# brafnras<-read.csv(paste(path,melanoma.meta,sep=""),header=TRUE)
```

```
# brafmss<-subset(brafnras, brafnras$BRAF=="1" & brafnras$include=="1" &
brafnras$MSS_Inc=="1")

melanoma.metamss<-"Final_Aggregate_braf_mss.csv"

brafmss<-read.csv(paste(path,melanoma.metamss,sep=""),header=TRUE)

brafmss.meta<- metagen(TE=brafmss$ln_hr_mss_braf,
seTE=brafmss$se_ln_hr_mss_braf, studlab=paste(brafmss$author, brafmss$Year,
sep=", "), sm="HR", level=0.95, level.comb=0.95, comb.fixed=TRUE,
comb.random=TRUE, hakn=FALSE, method.tau="DL", tau.preset=NULL, TE.tau=NULL,
method.bias="linreg", n.e=brafmss$braf_n, n.c=brafmss$braf_n_wt, title="HR_MSS
for BRAF", complab="", outclab="", label.e="BRAF", label.c="WT", label.left="",
label.right="", warn=TRUE)

print(brafmss.meta, sortvar=brafmss$Year, level=brafmss.meta$level,
level.comb=brafmss.meta$level.comb, comb.fixed=brafmss.meta$comb.fixed,
comb.random=brafmss.meta$comb.random, details=TRUE, ma=TRUE, digits=2)

win.metafile(filename = "Forest_brafmss.wmf", width = 10, height = 4.5,
pointsize = 12, restoreConsole = FALSE)
forest(brafmss.meta, smlab="HR MSS BRAF", sortvar=brafmss$Year)
dev.off()

# metabias(brafmss.meta, method.bias="rank", plotit=TRUE, correct=TRUE,
k.min=3)
metabias(brafmss.meta, method.bias="linreg", plotit=TRUE, correct=FALSE,
k.min=3)
# metabias(brafmss.meta, method.bias="mm", plotit=TRUE, correct=FALSE, k.min=3)

# metabias(brafmss.meta, method.bias="count", plotit=FALSE, correct=TRUE,
k.min=3)
# method.bias 'count' only defined for meta-analysis with binary outcome data
(function 'metabin')
# metabias(brafmss.meta, method.bias="score", plotit=TRUE, correct=FALSE,
k.min=3)
# method.bias 'score' only defined for meta-analysis with binary outcome data
(function 'metabin')
# metabias(brafmss.meta, method.bias="peters", plotit=FALSE, correct=FALSE,
k.min=3)
```

```
# method.bias 'peters' only defined for meta-analysis with binary outcome data
(function 'metabin')

metainf(brafmss.meta, pooled="random", sortvar=brafmss$Year,
level.comb=brafmss.meta$level.comb)

metacum(brafmss.meta, pooled="random", sortvar=brafmss$Year,
level.comb=brafmss.meta$level.comb)

win.metafile(filename = "Funnel_brafmss.wmf", width = 12, height = 9, pointsize
= 12, restoreConsole = FALSE)
funnel(brafmss.meta, comb.fixed=FALSE, comb.random=TRUE, level=0.95,
contour=c(0.9, 0.95, 0.99), col.contour=c("white", "grey", "lightgrey"), lwd=2,
cex=2, pch=16, studlab=TRUE, cex.studlab=0.75)
dev.off()

# win.metafile(filename = "Radial_brafmss.wmf", width = 9, height = 5,
pointsize = 12, restoreConsole = FALSE)
# radial(brafmss.meta, comb.fixed=TRUE, axes=TRUE, cex=1, pch=16, level=0.95)
# dev.off()

brafmss.trim<-trimfill(brafmss.meta)

print(brafmss.trim)

win.metafile(filename = "Funnel_brafmss.trim.wmf", width = 12, height = 9,
pointsize = 12, restoreConsole = FALSE)
funnel(brafmss.trim, comb.fixed=FALSE, comb.random=TRUE, level=0.95,
contour=c(0.9, 0.95, 0.99), col.contour=c("white", "grey", "lightgrey"), lwd=2,
cex=2, pch=16, studlab=TRUE, cex.studlab=0.75)
dev.off()




# path<-"Z:\\DATA\\"

# library("metafor")

# melanoma.metamss<-"Final_Aggregate_braf_mss.csv"
```

```
# brafmss<-read.csv(paste(path,melanoma.metamss,sep=""),header=TRUE)

# brafmss.meta<-rma.uni(yi=ln_hr_mss_braf, sei=se_ln_hr_mss_braf, data=brafmss, measure="GEN", slab=paste(brafmss$author, brafmss$Year, sep=", "), method="DL", knha=FALSE, level=95)

# regtest.rma(brafmss.meta, model="rma", predictor="ninv", ni=brafmss$braf_n_total)

# win.metafile(filename = "Radial_brafmss_2.wmf", width = 6, height = 5, pointsize = 12, restoreConsole = FALSE)
# radial(brafmss.meta, transf=exp, xlab="Inverse of Standard Error", zlab="Standardised Treatment Effect (z-score)")
# dev.off()





# ### BRAF OSMSS ###

#library("metafor")

#library("meta")

# melanoma.meta<-"Final_Aggregate.csv"

# brafnras<-read.csv(paste(path,melanoma.meta,sep=""),header=TRUE)

# brafosmss<-subset(brafnras, brafnras$BRAF=="1" & brafnras$include=="1" & brafnras$OSMSS_Inc=="1")

melanoma.metaosmss<-"Final_Aggregate_braf_osmss.csv"

brafosmss<-read.csv(paste(path,melanoma.metaosmss,sep=""),header=TRUE)

brafosmss.meta<- metagen(TE=brafosmss$ln_hr_osmss_braf, seTE=brafosmss$se_ln_hr_osmss_braf, studlab=paste(brafosmss$author, brafosmss$Year, sep=", "), sm="HR", level=0.95, level.comb=0.95,
```

```
comb.fixed=TRUE, comb.random=TRUE, hakn=FALSE, method.tau="DL",
tau.preset=NULL, TE.tau=NULL, method.bias="linreg", n.e=brafosmss$braf_n,
n.c=brafosmss$braf_n_wt, title="HR_osmss for BRAF", complab="", outclab="",
label.e="BRAF", label.c="WT", label.left="", label.right="", warn=TRUE)

print(brafosmss.meta, sortvar=brafosmss$Year, level=brafosmss.meta$level,
level.comb=brafosmss.meta$level.comb, comb.fixed=brafosmss.meta$comb.fixed,
comb.random=brafosmss.meta$comb.random, details=TRUE, ma=TRUE, digits=2)

win.metafile(filename = "Forest_brafosmss.wmf", width = 10, height = 5.5,
pointsize = 12, restoreConsole = FALSE)
forest(brafosmss.meta, smlab="HR OSMSS BRAF", sortvar=brafosmss$Year)
dev.off()

# metabias(brafosmss.meta, method.bias="rank", plotit=TRUE, correct=TRUE,
k.min=3)
metabias(brafosmss.meta, method.bias="linreg", plotit=TRUE, correct=FALSE,
k.min=3)
# metabias(brafosmss.meta, method.bias="mm", plotit=TRUE, correct=FALSE,
k.min=3)

# metabias(brafosmss.meta, method.bias="count", plotit=FALSE, correct=TRUE,
k.min=3)
# method.bias 'count' only defined for meta-analysis with binary outcome data
(function 'metabin')
# metabias(brafosmss.meta, method.bias="score", plotit=TRUE, correct=FALSE,
k.min=3)
# method.bias 'score' only defined for meta-analysis with binary outcome data
(function 'metabin')
# metabias(brafosmss.meta, method.bias="peters", plotit=FALSE, correct=FALSE,
k.min=3)
# method.bias 'peters' only defined for meta-analysis with binary outcome data
(function 'metabin')

metainf(brafosmss.meta, pooled="random", sortvar=brafosmss$Year,
level.comb=brafosmss.meta$level.comb)

metacum(brafosmss.meta, pooled="random", sortvar=brafosmss$Year,
level.comb=brafosmss.meta$level.comb)

win.metafile(filename = "Funnel_brafosmss.wmf", width = 12, height = 9,
pointsize = 12, restoreConsole = FALSE)
```

```
funnel(brafosmss.meta, comb.fixed=FALSE, comb.random=TRUE, level=0.95,
contour=c(0.9, 0.95, 0.99), col.contour=c("white", "grey", "lightgrey"), lwd=2,
cex=2, pch=16, studlab=TRUE, cex.studlab=0.75)
dev.off()

# win.metafile(filename = "Radial_brafosmss.wmf", width = 9, height = 5,
pointsize = 12, restoreConsole = FALSE)
# radial(brafosmss.meta, comb.fixed=TRUE, axes=TRUE, cex=1, pch=16, level=0.95)
# dev.off()

brafosmss.trim<-trimfill(brafosmss.meta)

print(brafosmss.trim)

win.metafile(filename = "Funnel_brafosmss.trim.wmf", width = 12, height = 9,
pointsize = 12, restoreConsole = FALSE)
funnel(brafosmss.trim, comb.fixed=FALSE, comb.random=TRUE, level=0.95,
contour=c(0.9, 0.95, 0.99), col.contour=c("white", "grey", "lightgrey"), lwd=2,
cex=2, pch=16, studlab=TRUE, cex.studlab=0.75)
dev.off()




# path<-"Z:\\DATA\\"

# library("metafor")

# melanoma.metaosmss<-"Final_Aggregate_braf_osmss.csv"

# brafosmss<-read.csv(paste(path,melanoma.metaosmss,sep=""),header=TRUE)

# brafosmss.meta<-rma.uni(yi=ln_hr_osmss_braf, sei=se_ln_hr_osmss_braf,
data=brafosmss, measure="GEN", slab=paste(brafosmss$author, brafosmss$Year,
sep=", "), method="DL", knha=FALSE, level=95)

# regtest.rma(brafosmss.meta, model="rma", predictor="ninv",
ni=brafosmss$braf_n_total)

# win.metafile(filename = "Radial_brafosmss_2.wmf", width = 6, height = 5,
pointsize = 12, restoreConsole = FALSE)
```

```
# radial(brafosmss.meta, transf=exp, xlab="Inverse of Standard Error",
zlab="Standardised Treatment Effect (z-score)")
# dev.off()


# ### NRAS ###


# ###NRAS DFS ###

# melanoma.meta<-"Final_Aggregate.csv"

# brafnras<-read.csv(paste(path,melanoma.meta,sep=""),header=TRUE)

# nrasdfs<-subset(brafnras, brafnras$NRAS=="1" & brafnras$include=="1" &
brafnras$DFS_Inc=="1")

melanoma.metadfs<-"Final_Aggregate_nras_dfs.csv"

nrasdfs<-read.csv(paste(path,melanoma.metadfs,sep=""),header=TRUE)

nrasdfs.meta<- metagen(TE=nrasdfs$ln_hr_dfs_nras,
seTE=nrasdfs$se_ln_hr_dfs_nras, studlab=paste(nrasdfs$author, nrasdfs$Year,
sep=", "), sm="HR", level=0.95, level.comb=0.95, comb.fixed=TRUE,
comb.random=TRUE, hakn=FALSE, method.tau="DL", tau.preset=NULL, TE.tau=NULL,
method.bias="linreg", n.e=nrasdfs$nras_n, n.c=nrasdfs$nras_n_wt, title="HR_DFS
for NRAS", complab="", outclab="", label.e="NRAS", label.c="WT", label.left="",
label.right="", warn=TRUE)

print(nrasdfs.meta, sortvar=nrasdfs$Year, level=nrasdfs.meta$level,
level.comb=nrasdfs.meta$level.comb, comb.fixed=nrasdfs.meta$comb.fixed,
comb.random=nrasdfs.meta$comb.random, details=TRUE, ma=TRUE, digits=2)

win.metafile(filename = "Forest_nrasdfs.wmf", width = 10, height = 4.5,
pointsize = 12, restoreConsole = FALSE)
forest(nrasdfs.meta, smlab="HR DFS NRAS", sortvar=nrasdfs$Year)
```

```
dev.off()

# metabias(nrasdfs.meta, method.bias="rank", plotit=TRUE, correct=TRUE,
k.min=3)
metabias(nrasdfs.meta, method.bias="linreg", plotit=TRUE, correct=FALSE,
k.min=3)
# metabias(nrasdfs.meta, method.bias="mm", plotit=TRUE, correct=FALSE, k.min=3)

# metabias(nrasdfs.meta, method.bias="count", plotit=FALSE, correct=TRUE,
k.min=3)
# method.bias 'count' only defined for meta-analysis with binary outcome data
(function 'metabin')
# metabias(nrasdfs.meta, method.bias="score", plotit=TRUE, correct=FALSE,
k.min=3)
# method.bias 'score' only defined for meta-analysis with binary outcome data
(function 'metabin')
# metabias(nrasdfs.meta, method.bias="peters", plotit=FALSE, correct=FALSE,
k.min=3)
# method.bias 'peters' only defined for meta-analysis with binary outcome data
(function 'metabin')

metainf(nrasdfs.meta, pooled="random", sortvar=nrasdfs$Year,
level.comb=nrasdfs.meta$level.comb)

metacum(nrasdfs.meta, pooled="random", sortvar=nrasdfs$Year,
level.comb=nrasdfs.meta$level.comb)

win.metafile(filename = "Funnel_nrasdfs.wmf", width = 12, height = 9, pointsize
= 12, restoreConsole = FALSE)
funnel(nrasdfs.meta, comb.fixed=FALSE, comb.random=TRUE, level=0.95,
contour=c(0.9, 0.95, 0.99), col.contour=c("white", "grey", "lightgrey"), lwd=2,
cex=2, pch=16, studlab=TRUE, cex.studlab=0.75)
dev.off()

# win.metafile(filename = "Radial_nrasdfs.wmf", width = 9, height = 5,
pointsize = 12, restoreConsole = FALSE)
# radial(nrasdfs.meta, comb.fixed=TRUE, axes=TRUE, cex=1, pch=16, level=0.95)
# dev.off()

nrasdfs.trim<-trimfill(nrasdfs.meta)

print(nrasdfs.trim)
```

```
win.metafile(filename = "Funnel_nrasdfs.trim.wmf", width = 12, height = 9,
pointsize = 12, restoreConsole = FALSE)
funnel(nrasdfs.trim, comb.fixed=FALSE, comb.random=TRUE, level=0.95,
contour=c(0.9, 0.95, 0.99), col.contour=c("white", "grey", "lightgrey"), lwd=2,
cex=2, pch=16, studlab=TRUE, cex.studlab=0.75)
dev.off()




# path<-"Z:\\DATA\\"

# library("metafor")

# melanoma.metadfs<-"Final_Aggregate_nras_dfs.csv"

# nrasdfs<-read.csv(paste(path,melanoma.metadfs,sep=""),header=TRUE)

# nrasdfs.meta<-rma.uni(yi=ln_hr_dfs_nras, sei=se_ln_hr_dfs_nras, data=nrasdfs,
measure="GEN", slab=paste(nrasdfs$author, nrasdfs$Year, sep=", "), method="DL",
knha=FALSE, level=95)

# regtest.rma(nrasdfs.meta, model="rma", predictor="ninv",
ni=nrasdfs$nras_n_total)

# win.metafile(filename = "Radial_nrasdfs_2.wmf", width = 6, height = 5,
pointsize = 12, restoreConsole = FALSE)
# radial(nrasdfs.meta, transf=exp, xlab="Inverse of Standard Error",
zlab="Standardised Treatment Effect (z-score)")
# dev.off()




# ### NRAS OS ###

# library("metafor")
```

```
# library("meta")

# melanoma.meta<-"Final_Aggregate.csv"

# nrasnras<-read.csv(paste(path,melanoma.meta,sep=""),header=TRUE)

# nrasos<-subset(nrasnras, nrasnras$NRAS=="1" & nrasnras$include=="1" &
nrasnras$OS_Inc=="1")

melanoma.metaos<-"Final_Aggregate_nras_os.csv"

nrasos<-read.csv(paste(path,melanoma.metaos,sep=""),header=TRUE)

nrasos.meta<- metagen(TE=nrasos$ln_hr_os_nras, seTE=nrasos$se_ln_hr_os_nras,
studlab=paste(nrasos$author, nrasos$Year, sep=", "), sm="HR", level=0.95,
level.comb=0.95, comb.fixed=TRUE, comb.random=TRUE, hakn=FALSE,
method.tau="DL", tau.preset=NULL, TE.tau=NULL, method.bias="linreg",
n.e=nrasos$nras_n, n.c=nrasos$nras_n_wt, title="HR_OS for NRAS", complab="",
outclab="", label.e="NRAS", label.c="WT", label.left="", label.right="",
warn=TRUE)

print(nrasos.meta, sortvar=nrasos$Year, level=nrasos.meta$level,
level.comb=nrasos.meta$level.comb, comb.fixed=nrasos.meta$comb.fixed,
comb.random=nrasos.meta$comb.random, details=TRUE, ma=TRUE, digits=2)

win.metafile(filename = "Forest_nrasos.wmf", width = 10, height = 4.5,
pointsize = 12, restoreConsole = FALSE)
forest(nrasos.meta, smlab="HR OS NRAS", sortvar=nrasos$Year)
dev.off()

# metabias(nrasos.meta, method.bias="rank", plotit=TRUE, correct=TRUE, k.min=3)
metabias(nrasos.meta, method.bias="linreg", plotit=TRUE, correct=FALSE,
k.min=3)
# metabias(nrasos.meta, method.bias="mm", plotit=TRUE, correct=FALSE, k.min=3)

# metabias(nrasos.meta, method.bias="count", plotit=FALSE, correct=TRUE,
k.min=3)
# method.bias 'count' only defined for meta-analysis with binary outcome data
(function 'metabin')
# metabias(nrasos.meta, method.bias="score", plotit=TRUE, correct=FALSE,
k.min=3)
```

```
# method.bias 'score' only defined for meta-analysis with binary outcome data
(function 'metabin')
# metabias(nrasos.meta, method.bias="peters", plotit=FALSE, correct=FALSE,
k.min=3)
# method.bias 'peters' only defined for meta-analysis with binary outcome data
(function 'metabin')

metainf(nrasos.meta, pooled="random", sortvar=nrasos$Year,
level.comb=nrasos.meta$level.comb)

metacum(nrasos.meta, pooled="random", sortvar=nrasos$Year,
level.comb=nrasos.meta$level.comb)

win.metafile(filename = "Funnel_nrasos.wmf", width = 12, height = 9, pointsize
= 12, restoreConsole = FALSE)
funnel(nrasos.meta, comb.fixed=FALSE, comb.random=TRUE, level=0.95,
contour=c(0.9, 0.95, 0.99), col.contour=c("white", "grey", "lightgrey"), lwd=2,
cex=2, pch=16, studlab=TRUE, cex.studlab=0.75)
dev.off()

# win.metafile(filename = "Radial_nrasos.wmf", width = 9, height = 5, pointsize
= 12, restoreConsole = FALSE)
# radial(nrasos.meta, comb.fixed=TRUE, axes=TRUE, cex=1, pch=16, level=0.95)
# dev.off()

nrasos.trim<-trimfill(nrasos.meta)

print(nrasos.trim)

win.metafile(filename = "Funnel_nrasos.trim.wmf", width = 12, height = 9,
pointsize = 12, restoreConsole = FALSE)
funnel(nrasos.trim, comb.fixed=FALSE, comb.random=TRUE, level=0.95,
contour=c(0.9, 0.95, 0.99), col.contour=c("white", "grey", "lightgrey"), lwd=2,
cex=2, pch=16, studlab=TRUE, cex.studlab=0.75)
dev.off()




# path<-"Z:\\DATA\\"
```

```
# library("metafor")

# melanoma.metaos<-"Final_Aggregate_nras_os.csv"

# nrasos<-read.csv(paste(path,melanoma.metaos,sep=""),header=TRUE)

# nrasos.meta<-rma.uni(yi=ln_hr_os_nras, sei=se_ln_hr_os_nras, data=nrasos,
measure="GEN", slab=paste(nrasos$author, nrasos$Year, sep=", "), method="DL",
knha=FALSE, level=95)

# regtest.rma(nrasos.meta, model="rma", predictor="ninv",
ni=nrasos$nras_n_total)

# win.metafile(filename = "Radial_nrasos_2.wmf", width = 6, height = 5,
pointsize = 12, restoreConsole = FALSE)
# radial(nrasos.meta, transf=exp, xlab="Inverse of Standard Error",
zlab="Standardised Treatment Effect (z-score)")
# dev.off()


# ### NRAS MSS ###

# library("metafor")

# library("meta")

# melanoma.meta<-"Final_Aggregate.csv"

# nrasnras<-read.csv(paste(path,melanoma.meta,sep=""),header=TRUE)

# nrasmss<-subset(nrasnras, nrasnras$NRAS=="1" & nrasnras$include=="1" &
nrasnras$MSS_Inc=="1")

melanoma.metamss<-"Final_Aggregate_nras_mss.csv"

nrasmss<-read.csv(paste(path,melanoma.metamss,sep=""),header=TRUE)
```

```
nrasmss.meta<- metagen(TE=nrasmss$ln_hr_mss_nras,
seTE=nrasmss$se_ln_hr_mss_nras, studlab=paste(nrasmss$author, nrasmss$Year,
sep=", "), sm="HR", level=0.95, level.comb=0.95, comb.fixed=TRUE,
comb.random=TRUE, hakn=FALSE, method.tau="DL", tau.preset=NULL, TE.tau=NULL,
method.bias="linreg", n.e=nrasmss$nras_n, n.c=nrasmss$nras_n_wt, title="HR_MSS
for NRAS", complab="", outclab="", label.e="NRAS", label.c="WT", label.left="",
label.right="", warn=TRUE)

print(nrasmss.meta, sortvar=nrasmss$Year, level=nrasmss.meta$level,
level.comb=nrasmss.meta$level.comb, comb.fixed=nrasmss.meta$comb.fixed,
comb.random=nrasmss.meta$comb.random, details=TRUE, ma=TRUE, digits=2)

win.metafile(filename = "Forest_nrasmss.wmf", width = 10, height = 4.5,
pointsize = 12, restoreConsole = FALSE)
forest(nrasmss.meta, smlab="HR MSS NRAS", sortvar=nrasmss$Year)
dev.off()

# metabias(nrasmss.meta, method.bias="rank", plotit=TRUE, correct=TRUE,
k.min=3)
metabias(nrasmss.meta, method.bias="linreg", plotit=TRUE, correct=FALSE,
k.min=3)
# metabias(nrasmss.meta, method.bias="mm", plotit=TRUE, correct=FALSE, k.min=3)

# metabias(nrasmss.meta, method.bias="count", plotit=FALSE, correct=TRUE,
k.min=3)
# method.bias 'count' only defined for meta-analysis with binary outcome data
(function 'metabin')
# metabias(nrasmss.meta, method.bias="score", plotit=TRUE, correct=FALSE,
k.min=3)
# method.bias 'score' only defined for meta-analysis with binary outcome data
(function 'metabin')
# metabias(nrasmss.meta, method.bias="peters", plotit=FALSE, correct=FALSE,
k.min=3)
# method.bias 'peters' only defined for meta-analysis with binary outcome data
(function 'metabin')

metainf(nrasmss.meta, pooled="random", sortvar=nrasmss$Year,
level.comb=nrasmss.meta$level.comb)

metacum(nrasmss.meta, pooled="random", sortvar=nrasmss$Year,
level.comb=nrasmss.meta$level.comb)
```

```
win.metafile(filename = "Funnel_nrasmss.wmf", width = 12, height = 9, pointsize
= 12, restoreConsole = FALSE)
funnel(nrasmss.meta, comb.fixed=FALSE, comb.random=TRUE, level=0.95,
contour=c(0.9, 0.95, 0.99), col.contour=c("white", "grey", "lightgrey"), lwd=2,
cex=2, pch=16, studlab=TRUE, cex.studlab=0.75)
dev.off()

# win.metafile(filename = "Radial_nrasmss.wmf", width = 9, height = 5,
pointsize = 12, restoreConsole = FALSE)
# radial(nrasmss.meta, comb.fixed=TRUE, axes=TRUE, cex=1, pch=16, level=0.95)
# dev.off()

nrasmss.trim<-trimfill(nrasmss.meta)

print(nrasmss.trim)

win.metafile(filename = "Funnel_nrasmss.trim.wmf", width = 12, height = 9,
pointsize = 12, restoreConsole = FALSE)
funnel(nrasmss.trim, comb.fixed=FALSE, comb.random=TRUE, level=0.95,
contour=c(0.9, 0.95, 0.99), col.contour=c("white", "grey", "lightgrey"), lwd=2,
cex=2, pch=16, studlab=TRUE, cex.studlab=0.75)
dev.off()




# path<-"Z:\\DATA\\"

# library("metafor")

# melanoma.metamss<-"Final_Aggregate_nras_mss.csv"

# nrasmss<-read.csv(paste(path,melanoma.metamss,sep=""),header=TRUE)

# nrasmss.meta<-rma.uni(yi=ln_hr_mss_nras, sei=se_ln_hr_mss_nras, data=nrasmss,
measure="GEN", slab=paste(nrasmss$author, nrasmss$Year, sep=", "), method="DL",
knha=FALSE, level=95)

# regtest.rma(nrasmss.meta, model="rma", predictor="ninv",
ni=nrasmss$nras_n_total)
```

```
# win.metafile(filename = "Radial_nrasmss_2.wmf", width = 6, height = 5,
pointsize = 12, restoreConsole = FALSE)
# radial(nrasmss.meta, transf=exp, xlab="Inverse of Standard Error",
zlab="Standardised Treatment Effect (z-score)")
# dev.off()
```

```
# ### NRAS OSMSS ###

# library("metafor")

# library("meta")

# melanoma.meta<-"Final_Aggregate.csv"

# nrasnras<-read.csv(paste(path,melanoma.meta,sep=""),header=TRUE)

# nrasosmss<-subset(nrasnras, nrasnras$NRAS=="1" & nrasnras$include=="1" &
nrasnras$OSMSS_Inc=="1")

melanoma.metaosmss<-"Final_Aggregate_nras_osmss.csv"

nrasosmss<-read.csv(paste(path,melanoma.metaosmss,sep=""),header=TRUE)

nrasosmss.meta<- metagen(TE=nrasosmss$ln_hr_osmss_nras,
seTE=nrasosmss$se_ln_hr_osmss_nras, studlab=paste(nrasosmss$author,
nrasosmss$Year, sep=", "), sm="HR", level=0.95, level.comb=0.95,
comb.fixed=TRUE, comb.random=TRUE, hakn=FALSE, method.tau="DL",
tau.preset=NULL, TE.tau=NULL, method.bias="linreg", n.e=nrasosmss$nras_n,
n.c=nrasosmss$nras_n_wt, title="HR_osmss for NRAS", complab="", outclab="",
label.e="NRAS", label.c="WT", label.left="", label.right="", warn=TRUE)

print(nrasosmss.meta, sortvar=nrasosmss$Year, level=nrasosmss.meta$level,
level.comb=nrasosmss.meta$level.comb, comb.fixed=nrasosmss.meta$comb.fixed,
comb.random=nrasosmss.meta$comb.random, details=TRUE, ma=TRUE, digits=2)
```

```
win.metafile(filename = "Forest_nrasosmss.wmf", width = 10, height = 4.5,
pointsize = 12, restoreConsole = FALSE)
forest(nrasosmss.meta, smlab="HR OSMSS NRAS", sortvar=nrasosmss$Year)
dev.off()

# metabias(nrasosmss.meta, method.bias="rank", plotit=TRUE, correct=TRUE,
k.min=3)
metabias(nrasosmss.meta, method.bias="linreg", plotit=TRUE, correct=FALSE,
k.min=3)
# metabias(nrasosmss.meta, method.bias="mm", plotit=TRUE, correct=FALSE,
k.min=3)

# metabias(nrasosmss.meta, method.bias="count", plotit=FALSE, correct=TRUE,
k.min=3)
# method.bias 'count' only defined for meta-analysis with binary outcome data
(function 'metabin')
# metabias(nrasosmss.meta, method.bias="score", plotit=TRUE, correct=FALSE,
k.min=3)
# method.bias 'score' only defined for meta-analysis with binary outcome data
(function 'metabin')
# metabias(nrasosmss.meta, method.bias="peters", plotit=FALSE, correct=FALSE,
k.min=3)
# method.bias 'peters' only defined for meta-analysis with binary outcome data
(function 'metabin')

metainf(nrasosmss.meta, pooled="random", sortvar=nrasosmss$Year,
level.comb=nrasosmss.meta$level.comb)

metacum(nrasosmss.meta, pooled="random", sortvar=nrasosmss$Year,
level.comb=nrasosmss.meta$level.comb)

win.metafile(filename = "Funnel_nrasosmss.wmf", width = 12, height = 9,
pointsize = 12, restoreConsole = FALSE)
funnel(nrasosmss.meta, comb.fixed=FALSE, comb.random=TRUE, level=0.95,
contour=c(0.9, 0.95, 0.99), col.contour=c("white", "grey", "lightgrey"), lwd=2,
cex=2, pch=16, studlab=TRUE, cex.studlab=0.75)
dev.off()

# win.metafile(filename = "Radial_nrasosmss.wmf", width = 9, height = 5,
pointsize = 12, restoreConsole = FALSE)
# radial(nrasosmss.meta, comb.fixed=TRUE, axes=TRUE, cex=1, pch=16, level=0.95)
```

```
# dev.off()

nrasosmss.trim<-trimfill(nrasosmss.meta)

print(nrasosmss.trim)

win.metafile(filename = "Funnel_nrasosmss.trim.wmf", width = 12, height = 9,
pointsize = 12, restoreConsole = FALSE)
funnel(nrasosmss.trim, comb.fixed=FALSE, comb.random=TRUE, level=0.95,
contour=c(0.9, 0.95, 0.99), col.contour=c("white", "grey", "lightgrey"), lwd=2,
cex=2, pch=16, studlab=TRUE, cex.studlab=0.75)
dev.off()




# path<-"Z:\\DATA\\"

# library("metafor")

# melanoma.metaosmss<-"Final_Aggregate_nras_osmss.csv"

# nrasosmss<-read.csv(paste(path,melanoma.metaosmss,sep=""),header=TRUE)

# nrasosmss.meta<-rma.uni(yi=ln_hr_osmss_nras, sei=se_ln_hr_osmss_nras,
data=nrasosmss, measure="GEN", slab=paste(nrasosmss$author, nrasosmss$Year,
sep=", "), method="DL", knha=FALSE, level=95)

# regtest.rma(nrasosmss.meta, model="rma", predictor="ninv",
ni=nrasosmss$nras_n_total)

# win.metafile(filename = "Radial_nrasosmss_2.wmf", width = 6, height = 5,
pointsize = 12, restoreConsole = FALSE)
# radial(nrasosmss.meta, transf=exp, xlab="Inverse of Standard Error",
zlab="Standardised Treatment Effect (z-score)")
# dev.off()
```

```
################################################################################
###################################################
###### The following is for package metafor only for Peters test and radial
graph ################################################
################################################################################
###################################################


path<-"Z:\\DATA\\"

sink("Melanoma_Metafor.txt", append=FALSE, split=TRUE)

library("metafor")



# ###BRAF DFS ###

melanoma.metadfs<-"Final_Aggregate_braf_dfs.csv"

brafdfs<-read.csv(paste(path,melanoma.metadfs,sep=""),header=TRUE)

brafdfs.meta<-rma.uni(yi=ln_hr_dfs_braf, sei=se_ln_hr_dfs_braf, data=brafdfs,
measure="GEN", slab=paste(brafdfs$author, brafdfs$Year, sep=", "), method="DL",
knha=FALSE, level=95)

regtest.rma(brafdfs.meta, model="rma", predictor="ninv",
ni=brafdfs$braf_n_total)

win.metafile(filename = "Radial_brafdfs_2.wmf", width = 6, height = 5,
pointsize = 12, restoreConsole = FALSE)
radial(brafdfs.meta, transf=exp, xlab="Inverse of Standard Error",
zlab="Standardised Treatment Effect (z-score)")
dev.off()
```

```
# ### BRAF OS ###

melanoma.metaos<-"Final_Aggregate_braf_os.csv"

brafos<-read.csv(paste(path,melanoma.metaos,sep=""),header=TRUE)

brafos.meta<-rma.uni(yi=ln_hr_os_braf, sei=se_ln_hr_os_braf, data=brafos,
measure="GEN", slab=paste(brafos$author, brafos$Year, sep=", "), method="DL",
knha=FALSE, level=95)

regtest.rma(brafos.meta, model="rma", predictor="ninv", ni=brafos$braf_n_total)

win.metafile(filename = "Radial_brafos_2.wmf", width = 6, height = 5, pointsize
= 12, restoreConsole = FALSE)
radial(brafos.meta, transf=exp, xlab="Inverse of Standard Error",
zlab="Standardised Treatment Effect (z-score)")
dev.off()




# ### BRAF MSS ###

melanoma.metamss<-"Final_Aggregate_braf_mss.csv"

brafmss<-read.csv(paste(path,melanoma.metamss,sep=""),header=TRUE)

brafmss.meta<-rma.uni(yi=ln_hr_mss_braf, sei=se_ln_hr_mss_braf, data=brafmss,
measure="GEN", slab=paste(brafmss$author, brafmss$Year, sep=", "), method="DL",
knha=FALSE, level=95)

regtest.rma(brafmss.meta, model="rma", predictor="ninv",
ni=brafmss$braf_n_total)

win.metafile(filename = "Radial_brafmss_2.wmf", width = 6, height = 5,
pointsize = 12, restoreConsole = FALSE)
radial(brafmss.meta, transf=exp, xlab="Inverse of Standard Error",
zlab="Standardised Treatment Effect (z-score)")
dev.off()
```

```
# ### BRAF OSMSS ###

melanoma.metaosmss<-"Final_Aggregate_braf_osmss.csv"

brafosmss<-read.csv(paste(path,melanoma.metaosmss,sep=""),header=TRUE)

brafosmss.meta<-rma.uni(yi=ln_hr_osmss_braf, sei=se_ln_hr_osmss_braf,
data=brafosmss, measure="GEN", slab=paste(brafosmss$author, brafosmss$Year,
sep=", "), method="DL", knha=FALSE, level=95)

regtest.rma(brafosmss.meta, model="rma", predictor="ninv",
ni=brafosmss$braf_n_total)

win.metafile(filename = "Radial_brafosmss_2.wmf", width = 6, height = 5,
pointsize = 12, restoreConsole = FALSE)
radial(brafosmss.meta, transf=exp, xlab="Inverse of Standard Error",
zlab="Standardised Treatment Effect (z-score)")
dev.off()




# ### NRAS ###


# ###NRAS DFS ###

melanoma.metadfs<-"Final_Aggregate_nras_dfs.csv"

nrasdfs<-read.csv(paste(path,melanoma.metadfs,sep=""),header=TRUE)
```

```
nrasdfs.meta<-rma.uni(yi=ln_hr_dfs_nras, sei=se_ln_hr_dfs_nras, data=nrasdfs,
measure="GEN", slab=paste(nrasdfs$author, nrasdfs$Year, sep=", "), method="DL",
knha=FALSE, level=95)

regtest.rma(nrasdfs.meta, model="rma", predictor="ninv",
ni=nrasdfs$nras_n_total)

win.metafile(filename = "Radial_nrasdfs_2.wmf", width = 6, height = 5,
pointsize = 12, restoreConsole = FALSE)
radial(nrasdfs.meta, transf=exp, xlab="Inverse of Standard Error",
zlab="Standardised Treatment Effect (z-score)")
dev.off()




# ### NRAS OS ###

melanoma.metaos<-"Final_Aggregate_nras_os.csv"

nrasos<-read.csv(paste(path,melanoma.metaos,sep=""),header=TRUE)

nrasos.meta<-rma.uni(yi=ln_hr_os_nras, sei=se_ln_hr_os_nras, data=nrasos,
measure="GEN", slab=paste(nrasos$author, nrasos$Year, sep=", "), method="DL",
knha=FALSE, level=95)

regtest.rma(nrasos.meta, model="rma", predictor="ninv", ni=nrasos$nras_n_total)

win.metafile(filename = "Radial_nrasos_2.wmf", width = 6, height = 5, pointsize
= 12, restoreConsole = FALSE)
radial(nrasos.meta, transf=exp, xlab="Inverse of Standard Error",
zlab="Standardised Treatment Effect (z-score)")
dev.off()
```

```
# ### NRAS MSS ###

melanoma.metamss<-"Final_Aggregate_nras_mss.csv"

nrasmss<-read.csv(paste(path,melanoma.metamss,sep=""),header=TRUE)

nrasmss.meta<-rma.uni(yi=ln_hr_mss_nras, sei=se_ln_hr_mss_nras, data=nrasmss,
measure="GEN", slab=paste(nrasmss$author, nrasmss$Year, sep=", "), method="DL",
knha=FALSE, level=95)

regtest.rma(nrasmss.meta, model="rma", predictor="ninv",
ni=nrasmss$nras_n_total)

win.metafile(filename = "Radial_nrasmss_2.wmf", width = 6, height = 5,
pointsize = 12, restoreConsole = FALSE)
radial(nrasmss.meta, transf=exp, xlab="Inverse of Standard Error",
zlab="Standardised Treatment Effect (z-score)")
dev.off()




# ### NRAS OSMSS ###

melanoma.metaosmss<-"Final_Aggregate_nras_osmss.csv"

nrasosmss<-read.csv(paste(path,melanoma.metaosmss,sep=""),header=TRUE)

nrasosmss.meta<-rma.uni(yi=ln_hr_osmss_nras, sei=se_ln_hr_osmss_nras,
data=nrasosmss, measure="GEN", slab=paste(nrasosmss$author, nrasosmss$Year,
sep=", "), method="DL", knha=FALSE, level=95)

regtest.rma(nrasosmss.meta, model="rma", predictor="ninv",
ni=nrasosmss$nras_n_total)

win.metafile(filename = "Radial_nrasosmss_2.wmf", width = 6, height = 5,
pointsize = 12, restoreConsole = FALSE)
```

```
radial(nrasosmss.meta, transf=exp, xlab="Inverse of Standard Error",
zlab="Standardised Treatment Effect (z-score)")
dev.off()





##################################################################################
###################################################
####  Template that was used to write the above code - Do not delete
##############################################################
##################################################################################
###################################################

library("metafor")

library("meta")

# melanoma.meta<-"Final_Aggregate.csv"

# nrasnras<-read.csv(paste(path,melanoma.meta,sep=""),header=TRUE)

# nrasdfs<-subset(nrasnras, nrasnras$NRAS=="1" & nrasnras$include=="1" &
nrasnras$DFS_Inc=="1")

melanoma.metadfs<-"Final_Aggregate_nras_dfs.csv"

nrasdfs<-read.csv(paste(path,melanoma.metadfs,sep=""),header=TRUE)

nrasdfs.meta<- metagen(TE=nrasdfs$ln_hr_dfs_nras,
seTE=nrasdfs$se_ln_hr_dfs_nras, studlab=paste(nrasdfs$author, nrasdfs$Year,
sep=", "), sm="HR", level=0.95, level.comb=0.95, comb.fixed=TRUE,
comb.random=TRUE, hakn=FALSE, method.tau="DL", tau.preset=NULL, TE.tau=NULL,
method.bias="linreg", n.e=nrasdfs$nras_n, n.c=nrasdfs$nras_n_wt, title="HR_DFS
for NRAS", complab="", outclab="", label.e="NRAS", label.c="WT", label.left="",
label.right="", warn=TRUE)

print(nrasdfs.meta, sortvar=nrasdfs$Year, level=nrasdfs.meta$level,
level.comb=nrasdfs.meta$level.comb, comb.fixed=nrasdfs.meta$comb.fixed,
comb.random=nrasdfs.meta$comb.random, details=TRUE, ma=TRUE, digits=2)
```

```
win.metafile(filename = "Forest_nrasdfs.wmf", width = 10, height = 4.5,
pointsize = 12, restoreConsole = FALSE)
forest(nrasdfs.meta, smlab="HR DFS NRAS", sortvar=nrasdfs$Year)
dev.off()

# metabias(nrasdfs.meta, method.bias="rank", plotit=TRUE, correct=TRUE,
k.min=3)
metabias(nrasdfs.meta, method.bias="linreg", plotit=TRUE, correct=FALSE,
k.min=3)
# metabias(nrasdfs.meta, method.bias="mm", plotit=TRUE, correct=FALSE, k.min=3)

# metabias(nrasdfs.meta, method.bias="count", plotit=FALSE, correct=TRUE,
k.min=3)
# method.bias 'count' only defined for meta-analysis with binary outcome data
(function 'metabin')
# metabias(nrasdfs.meta, method.bias="score", plotit=TRUE, correct=FALSE,
k.min=3)
# method.bias 'score' only defined for meta-analysis with binary outcome data
(function 'metabin')
# metabias(nrasdfs.meta, method.bias="peters", plotit=FALSE, correct=FALSE,
k.min=3)
# method.bias 'peters' only defined for meta-analysis with binary outcome data
(function 'metabin')

metainf(nrasdfs.meta, pooled="random", sortvar=nrasdfs$Year,
level.comb=nrasdfs.meta$level.comb)

metacum(nrasdfs.meta, pooled="random", sortvar=nrasdfs$Year,
level.comb=nrasdfs.meta$level.comb)

win.metafile(filename = "Funnel_nrasdfs.wmf", width = 12, height = 9, pointsize
= 12, restoreConsole = FALSE)
funnel(nrasdfs.meta, comb.fixed=FALSE, comb.random=TRUE, level=0.95,
contour=c(0.9, 0.95, 0.99), col.contour=c("white", "grey", "lightgrey"), lwd=2,
cex=2, pch=16, studlab=TRUE, cex.studlab=0.75)
dev.off()

# win.metafile(filename = "Radial_nrasdfs.wmf", width = 9, height = 5,
pointsize = 12, restoreConsole = FALSE)
# radial(nrasdfs.meta, comb.fixed=TRUE, axes=TRUE, cex=1, pch=16, level=0.95)
# dev.off()
```

```
nrasdfs.trim<-trimfill(nrasdfs.meta)

print(nrasdfs.trim)

win.metafile(filename = "Funnel_nrasdfs.trim.wmf", width = 12, height = 9,
pointsize = 12, restoreConsole = FALSE)
funnel(nrasdfs.trim, comb.fixed=FALSE, comb.random=TRUE, level=0.95,
contour=c(0.9, 0.95, 0.99), col.contour=c("white", "grey", "lightgrey"), lwd=2,
cex=2, pch=16, studlab=TRUE, cex.studlab=0.75)
dev.off()




path<-"Z:\\DATA\\"

library("metafor")

melanoma.metadfs<-"Final_Aggregate_nras_dfs.csv"

nrasdfs<-read.csv(paste(path,melanoma.metadfs,sep=""),header=TRUE)

nrasdfs.meta<-rma.uni(yi=ln_hr_dfs_nras, sei=se_ln_hr_dfs_nras, data=nrasdfs,
measure="GEN", slab=paste(nrasdfs$author, nrasdfs$Year, sep=", "), method="DL",
knha=FALSE, level=95)

regtest.rma(nrasdfs.meta, model="rma", predictor="ninv",
ni=nrasdfs$nras_n_total)

win.metafile(filename = "Radial_nrasdfs_2.wmf", width = 6, height = 5,
pointsize = 12, restoreConsole = FALSE)
radial(nrasdfs.meta, transf=exp, xlab="Inverse of Standard Error",
zlab="Standardised Treatment Effect (z-score)")
dev.off()




##############################################################################
##############################################
```

```
### Meta-regression of the meta-analysis only for statistical results
########################################################
### No need for variable braf_exons_2 for mss as they are all exons 15
#######################################################
###############################################################################
###############################################

path<-"Z:\\DATA\\"

sink("Melanoma_Meta_Metaregression.txt", append=FALSE, split=TRUE)

library("metafor")

melanoma.metaos<-"Final_Aggregate_braf_os.csv"

brafos<-read.csv(paste(path,melanoma.metaos,sep=""),header=TRUE)

metareg1a<-rma.uni(yi=ln_hr_os_braf, sei=se_ln_hr_os_braf, data=brafos,
measure="GEN", slab=paste(brafos$author, brafos$Year, sep=", "), method="DL",
knha=FALSE, level=95, mods=~factor(time_from)-1)

print.rma.uni(metareg1a, showfit=TRUE, signif.legend=FALSE)

metareg1b<-rma.uni(yi=ln_hr_os_braf, sei=se_ln_hr_os_braf, data=brafos,
measure="GEN", slab=paste(brafos$author, brafos$Year, sep=", "), method="DL",
knha=FALSE, level=95, mods=~factor(time_from))

print.rma.uni(metareg1b, showfit=TRUE, signif.legend=FALSE)



# example # metareg8<-rma.uni(yi=ln_hr_os_braf, sei=se_ln_hr_os_braf,
data=brafos, measure="GEN", slab=paste(brafos$author, brafos$Year, sep=", "),
method="DL", knha=FALSE, # example # level=95, # example #
mods=~factor(Time_From)*(DESIGN))

# example # print.rma.uni(all8, showfit=TRUE, signif.legend=FALSE)


############################ with -1 we get all factors and no intercept
############################################
```

```
path<-"Z:\\DATA\\"

library("metafor")

library("meta")

melanoma.metaos<-"Final_Aggregate_braf_os.csv"

brafos<-read.csv(paste(path,melanoma.metaos,sep=""),header=TRUE)

brafos.meta<- metagen(TE=brafos$ln_hr_os_braf, seTE=brafos$se_ln_hr_os_braf,
studlab=paste(brafos$author, brafos$Year, sep=", "), sm="HR", level=0.95,
level.comb=0.95, comb.fixed=TRUE, comb.random=TRUE, hakn=FALSE,
method.tau="DL", tau.preset=NULL, TE.tau=NULL, method.bias="linreg",
n.e=brafos$braf_n, n.c=brafos$braf_n_wt, title="HR_OS for BRAF", complab="",
outclab="", label.e="BRAF", label.c="WT", label.left="", label.right="",
warn=TRUE)

metareg(~brafos$time_from, data=brafos.meta)

metareg(~(brafos$time_from)-1, data=brafos.meta)




metareg(~brafos$Continent, data=brafos.meta)

metareg(~brafos$design, data=brafos.meta)

metareg(~brafos$fixation, data=brafos.meta)

metareg(~brafos$Specimen_Type, data=brafos.meta)
```

```
metareg(~brafos$braf_exons_2, data=brafos.meta)

metareg(~brafos$IOR, data=brafos.meta)

metareg(~brafos$SOR, data=brafos.meta)

metareg(~brafos$other_risk, data=brafos.meta)

metareg(~brafos$data_from, data=brafos.meta)



metareg(~(brafos$only_met_patients), data=brafos.meta)
metareg(~(brafos$only_met_patients)-1, data=brafos.meta)


metareg(~brafos$only_met_patients+brafos$time_from, data=brafos.meta)

metareg(~brafos$only_met_patients+brafos$Specimen_Type, data=brafos.meta)
metareg(~(brafos$only_met_patients+brafos$Specimen_Type)-1, data=brafos.meta)

metareg(~brafos$only_met_patients+brafos$data_from, data=brafos.meta)

###############################################################################
################################################


#############################################################################

####     Code for Cox PH for the whole IPD file    ##########################

#############################################################################



path<-"Z:\\DATA\\"

sink("Melanoma_Meta_IPD.txt", append=FALSE, split=TRUE)

library ("plyr")
```

```
library ("survival")

melanoma.metaipd<-"Final_IPD_no Deich and Demu.csv"

brafnrasipd<-read.csv(paste(path,melanoma.metaipd,sep=""),header=TRUE)


# Summary statistics of IPD data for BRAF and NRAS ######################


ddply(brafnrasipd, .(NRAS), summary)

ddply(brafnrasipd, .(BRAF), summary)



# DFS, OS and MSS for BRAF and NRAS, from IPD data ######################

brafnrasipdBRAF.dfs <- coxph(Surv(DFS, DFSstatus)~BRAF, data=brafnrasipd)

summary(brafnrasipdBRAF.dfs)

brafnrasipdBRAF.os <- coxph(Surv(OS, OSstatus)~BRAF, data=brafnrasipd)

summary(brafnrasipdBRAF.os)

brafnrasipdBRAF.mss <- coxph(Surv(MSS, MSSstatus)~BRAF, data=brafnrasipd)

summary(brafnrasipdBRAF.mss)


brafnrasipdNRAS.dfs <- coxph(Surv(DFS, DFSstatus)~NRAS, data=brafnrasipd)

summary(brafnrasipdNRAS.dfs)

brafnrasipdNRAS.os <- coxph(Surv(OS, OSstatus)~NRAS, data=brafnrasipd)

summary(brafnrasipdNRAS.os)

brafnrasipdNRAS.mss <- coxph(Surv(MSS, MSSstatus)~NRAS, data=brafnrasipd)
```

```
summary(brafnrasipdNRAS.mss)


# Remember in IPD data the OSstatus includes the MSSstatus anyway, so there is
no reason for both combined #



##### One stage Cox analysis stratified by article
###############################################################################
#####################



brafnrasipdBRAF.dfs <- coxph(Surv(DFS, DFSstatus)~BRAF+strata(Author),
data=brafnrasipd)

summary(brafnrasipdBRAF.dfs)

brafnrasipdBRAF.os <- coxph(Surv(OS, OSstatus)~BRAF+strata(Author),
data=brafnrasipd)

summary(brafnrasipdBRAF.os)

brafnrasipdBRAF.mss <- coxph(Surv(MSS, MSSstatus)~BRAF+strata(Author),
data=brafnrasipd)

summary(brafnrasipdBRAF.mss)


brafnrasipdNRAS.dfs <- coxph(Surv(DFS, DFSstatus)~NRAS+strata(Author),
data=brafnrasipd)

summary(brafnrasipdNRAS.dfs)

brafnrasipdNRAS.os <- coxph(Surv(OS, OSstatus)~NRAS+strata(Author),
data=brafnrasipd)

summary(brafnrasipdNRAS.os)
```

```
brafnrasipdNRAS.mss <- coxph(Surv(MSS, MSSstatus)~NRAS+strata(Author),
data=brafnrasipd)

summary(brafnrasipdNRAS.mss)


# Remember in IPD data the OSstatus includes the MSSstatus anyway, so there is
no reason for both combined ############################################

##### strata and some factors
###############################################################################
##########################################



brafnrasipdBRAF.os <- coxph(Surv(OS,
OSstatus)~BRAF+Sex+Age+Primary+as.numeric(Thickness)+Clark+Stage+Type+Continent
+Site+Ulceration+strata(Author), data=brafnrasipd)
summary(brafnrasipdBRAF.os)

brafnrasipdBRAF.os <- coxph(Surv(OS, OSstatus)~BRAF+Stage+strata(Author),
data=brafnrasipd)
summary(brafnrasipdBRAF.os)


brafnrasipdBRAF.os <- coxph(Surv(OS,
OSstatus)~BRAF+Sex+Age+Stage+strata(Author), data=brafnrasipd)
summary(brafnrasipdBRAF.os)

brafnrasipdBRAF.os <- coxph(Surv(OS,
OSstatus)~BRAF+Stage+BRAF*Stage+strata(Author), data=brafnrasipd)
summary(brafnrasipdBRAF.os)

brafnrasipdBRAF.os <- coxph(Surv(OS,
OSstatus)~BRAF+Stage+Sex+Age+as.numeric(Thickness)+strata(Author),
data=brafnrasipd)
summary(brafnrasipdBRAF.os)
```

```
########################################
########################################
######################################## strata and BRAF per stage
######################################################


brafnrasipdBRAF.os <- coxph(Surv(OS, OSstatus)~BRAF+strata(Author),
data=brafnrasipd, subset=(brafnrasipd$Stage=="IV"))

summary(brafnrasipdBRAF.os)


brafnrasipdBRAF.os <- coxph(Surv(OS, OSstatus)~BRAF+Age+strata(Author),
data=brafnrasipd, subset=(brafnrasipd$Stage=="IV"))

summary(brafnrasipdBRAF.os)


brafnrasipdBRAF.os <- coxph(Surv(OS, OSstatus)~BRAF+Age+Sex+strata(Author),
data=brafnrasipd, subset=(brafnrasipd$Stage=="IV"))

summary(brafnrasipdBRAF.os)


brafnrasipdBRAF.os <- coxph(Surv(OS,
OSstatus)~BRAF+Age+Sex+BRAF*Sex+strata(Author), data=brafnrasipd,
subset=(brafnrasipd$Stage=="IV"))

summary(brafnrasipdBRAF.os)


###### without strata as above ############

brafnrasipdBRAF.os <- coxph(Surv(OS, OSstatus)~BRAF, data=brafnrasipd,
subset=(brafnrasipd$Stage=="IV"))

summary(brafnrasipdBRAF.os)
```

```
brafnrasipdBRAF.os <- coxph(Surv(OS, OSstatus)~BRAF+Age, data=brafnrasipd,
subset=(brafnrasipd$Stage=="IV"))

summary(brafnrasipdBRAF.os)


brafnrasipdBRAF.os <- coxph(Surv(OS, OSstatus)~BRAF+Age+Sex, data=brafnrasipd,
subset=(brafnrasipd$Stage=="IV"))

summary(brafnrasipdBRAF.os)


brafnrasipdBRAF.os <- coxph(Surv(OS, OSstatus)~BRAF+strata(Author),
data=brafnrasipd, subset=(brafnrasipd$Stage=="III"))

summary(brafnrasipdBRAF.os)


brafnrasipdBRAF.os <- coxph(Surv(OS, OSstatus)~BRAF+Age+strata(Author),
data=brafnrasipd, subset=(brafnrasipd$Stage=="III"))

summary(brafnrasipdBRAF.os)


brafnrasipdBRAF.os <- coxph(Surv(OS, OSstatus)~BRAF+Age+Sex+strata(Author),
data=brafnrasipd, subset=(brafnrasipd$Stage=="III"))

summary(brafnrasipdBRAF.os)
```

```
brafnrasipdBRAF.os <- coxph(Surv(OS, OSstatus)~BRAF+strata(Author),
data=brafnrasipd, subset=(brafnrasipd$Stage=="II"))

summary(brafnrasipdBRAF.os)


brafnrasipdBRAF.os <- coxph(Surv(OS, OSstatus)~BRAF+Age+strata(Author),
data=brafnrasipd, subset=(brafnrasipd$Stage=="II"))

summary(brafnrasipdBRAF.os)


brafnrasipdBRAF.os <- coxph(Surv(OS, OSstatus)~BRAF+Age+Sex+strata(Author),
data=brafnrasipd, subset=(brafnrasipd$Stage=="II"))

summary(brafnrasipdBRAF.os)


brafnrasipdBRAF.os <- coxph(Surv(OS, OSstatus)~BRAF+strata(Author),
data=brafnrasipd, subset=(brafnrasipd$Stage=="I"))

summary(brafnrasipdBRAF.os)


brafnrasipdBRAF.os <- coxph(Surv(OS, OSstatus)~BRAF+Age+strata(Author),
data=brafnrasipd, subset=(brafnrasipd$Stage=="I"))

summary(brafnrasipdBRAF.os)


brafnrasipdBRAF.os <- coxph(Surv(OS, OSstatus)~BRAF+Age+Sex+strata(Author),
data=brafnrasipd, subset=(brafnrasipd$Stage=="I"))

summary(brafnrasipdBRAF.os)
```

```
brafnrasipdBRAF.os <- coxph(Surv(OS, OSstatus)~BRAF+strata(Author),
data=brafnrasipd, subset=(brafnrasipd$Stage=="UN"))

summary(brafnrasipdBRAF.os)


brafnrasipdBRAF.os <- coxph(Surv(OS, OSstatus)~BRAF+Age+strata(Author),
data=brafnrasipd, subset=(brafnrasipd$Stage=="UN"))

summary(brafnrasipdBRAF.os)


brafnrasipdBRAF.os <- coxph(Surv(OS, OSstatus)~BRAF+Age+Sex+strata(Author),
data=brafnrasipd, subset=(brafnrasipd$Stage=="UN"))

summary(brafnrasipdBRAF.os)
```

```
brafnrasipdBRAF.os <- coxph(Surv(OS,
OSstatus)~as.numeric(Thickness)+strata(Author), data=brafnrasipd)
summary(brafnrasipdBRAF.os)


brafnrasipdBRAF.os <- coxph(Surv(OS,
OSstatus)~BRAF+as.numeric(Thickness)+strata(Author), data=brafnrasipd)
summary(brafnrasipdBRAF.os)
```

```
brafnrasipdBRAF.os <- coxph(Surv(OS,
OSstatus)~BRAF+as.numeric(Thickness)+Stage+strata(Author), data=brafnrasipd)
summary(brafnrasipdBRAF.os)


brafnrasipdBRAF.os <- coxph(Surv(OS,
OSstatus)~BRAF+as.numeric(Thickness)+strata(Author), data=brafnrasipd,
subset=(brafnrasipd$Stage=="IV"))
summary(brafnrasipdBRAF.os)


brafnrasipdBRAF.os <- coxph(Surv(OS,
OSstatus)~BRAF+as.numeric(Thickness)+Sex+Age+strata(Author), data=brafnrasipd,
subset=(brafnrasipd$Stage=="IV"))
summary(brafnrasipdBRAF.os)


###############################################################################
########################################################################


# Cox for all the variables for IPD data - There are not enough observations to
combine all those ######################################################

brafnrasipdBRAF.dfs <- coxph(Surv(DFS,
DFSstatus)~BRAF+Sex+Age+Primary+Thickness+Clark+Stage+Type+Continent+Site+Ulcer
ation, data=brafnrasipd)
summary(brafnrasipdBRAF.dfs)
```

```
brafnrasipdBRAF.os <- coxph(Surv(OS,
OSstatus)~BRAF+Sex+Age+Primary+Thickness+Clark+Stage+Type+Continent+Site+Ulcera
tion, data=brafnrasipd)
summary(brafnrasipdBRAF.os)

brafnrasipdNRAS.dfs <- coxph(Surv(DFS,
DFSstatus)~NRAS+Sex+Age+Primary+Thickness+Clark+Stage+Type+Continent+Site+Ulcer
ation, data=brafnrasipd)
summary(brafnrasipdNRAS.dfs)

brafnrasipdNRAS.os <- coxph(Surv(OS,
OSstatus)~NRAS+Sex+Age+Primary+Thickness+Clark+Stage+Type+Continent+Site+Ulcera
tion, data=brafnrasipd)
summary(brafnrasipdNRAS.os)



# Cox for known co-founders and stage for IPD data - This is important - Only
stage and age seem to matter for overall survival
##################################

brafnrasipdBRAF.dfs <- coxph(Surv(DFS, DFSstatus)~BRAF+Sex+Age+Stage,
data=brafnrasipd)
summary(brafnrasipdBRAF.dfs)

brafnrasipdBRAF.os <- coxph(Surv(OS, OSstatus)~BRAF+Sex+Age+Stage,
data=brafnrasipd)
summary(brafnrasipdBRAF.os)

brafnrasipdNRAS.dfs <- coxph(Surv(DFS, DFSstatus)~NRAS+Sex+Age+Stage,
data=brafnrasipd)
summary(brafnrasipdNRAS.dfs)

brafnrasipdNRAS.os <- coxph(Surv(OS, OSstatus)~NRAS+Sex+Age+Stage,
data=brafnrasipd)
summary(brafnrasipdNRAS.os)



# Cox for BRAF and NRAS and stage for IPD data
##############################################################
```

```
brafnrasipdBRAF.dfs <- coxph(Surv(DFS, DFSstatus)~BRAF+Stage, data=brafnrasipd)

summary(brafnrasipdBRAF.dfs)

brafnrasipdBRAF.os <- coxph(Surv(OS, OSstatus)~BRAF+Stage, data=brafnrasipd)

summary(brafnrasipdBRAF.os)

brafnrasipdBRAF.mss <- coxph(Surv(MSS, MSSstatus)~BRAF+Stage, data=brafnrasipd)

summary(brafnrasipdBRAF.mss)


brafnrasipdNRAS.dfs <- coxph(Surv(DFS, DFSstatus)~NRAS+Stage, data=brafnrasipd)

summary(brafnrasipdNRAS.dfs)

brafnrasipdNRAS.os <- coxph(Surv(OS, OSstatus)~NRAS+Stage, data=brafnrasipd)

summary(brafnrasipdNRAS.os)

brafnrasipdNRAS.mss <- coxph(Surv(MSS, MSSstatus)~NRAS+Stage, data=brafnrasipd)

summary(brafnrasipdNRAS.mss)

################################################################################
################################


brafnrasipdBRAF.os <- coxph(Surv(OS, OSstatus)~BRAF+Stage+BRAF*Stage,
data=brafnrasipd)

summary(brafnrasipdBRAF.os)



brafnrasipdBRAF.os <- coxph(Surv(OS, OSstatus)~BRAF, data=brafnrasipd,
subset=(brafnrasipd$Stage=="IV"))
```

```
summary(brafnrasipdBRAF.os)


brafnrasipdBRAF.os <- coxph(Surv(OS, OSstatus)~BRAF+Age, data=brafnrasipd,
subset=(brafnrasipd$Stage=="IV"))

summary(brafnrasipdBRAF.os)


brafnrasipdBRAF.os <- coxph(Surv(OS, OSstatus)~BRAF+Age+Sex, data=brafnrasipd,
subset=(brafnrasipd$Stage=="IV"))

summary(brafnrasipdBRAF.os)


brafnrasipdBRAF.os <- coxph(Surv(OS, OSstatus)~BRAF+Age+Sex+BRAF*Sex,
data=brafnrasipd, subset=(brafnrasipd$Stage=="IV"))

summary(brafnrasipdBRAF.os)




brafnrasipdBRAF.os <- coxph(Surv(OS, OSstatus)~BRAF, data=brafnrasipd,
subset=(brafnrasipd$Stage=="III"))

summary(brafnrasipdBRAF.os)


brafnrasipdBRAF.os <- coxph(Surv(OS, OSstatus)~BRAF+Age, data=brafnrasipd,
subset=(brafnrasipd$Stage=="III"))

summary(brafnrasipdBRAF.os)


brafnrasipdBRAF.os <- coxph(Surv(OS, OSstatus)~BRAF+Age+Sex, data=brafnrasipd,
subset=(brafnrasipd$Stage=="III"))

summary(brafnrasipdBRAF.os)
```

```
brafnrasipdBRAF.os <- coxph(Surv(OS, OSstatus)~BRAF, data=brafnrasipd,
subset=(brafnrasipd$Stage=="II"))

summary(brafnrasipdBRAF.os)


brafnrasipdBRAF.os <- coxph(Surv(OS, OSstatus)~BRAF+Age, data=brafnrasipd,
subset=(brafnrasipd$Stage=="II"))

summary(brafnrasipdBRAF.os)


brafnrasipdBRAF.os <- coxph(Surv(OS, OSstatus)~BRAF+Age+Sex, data=brafnrasipd,
subset=(brafnrasipd$Stage=="II"))

summary(brafnrasipdBRAF.os)


brafnrasipdBRAF.os <- coxph(Surv(OS, OSstatus)~BRAF, data=brafnrasipd,
subset=(brafnrasipd$Stage=="I"))

summary(brafnrasipdBRAF.os)


brafnrasipdBRAF.os <- coxph(Surv(OS, OSstatus)~BRAF+Age, data=brafnrasipd,
subset=(brafnrasipd$Stage=="I"))

summary(brafnrasipdBRAF.os)


brafnrasipdBRAF.os <- coxph(Surv(OS, OSstatus)~BRAF+Age+Sex, data=brafnrasipd,
subset=(brafnrasipd$Stage=="I"))
```

```
summary(brafnrasipdBRAF.os)

brafnrasipdBRAF.os <- coxph(Surv(OS, OSstatus)~BRAF, data=brafnrasipd,
subset=(brafnrasipd$Stage=="UN"))

summary(brafnrasipdBRAF.os)

brafnrasipdBRAF.os <- coxph(Surv(OS, OSstatus)~BRAF+Age, data=brafnrasipd,
subset=(brafnrasipd$Stage=="I"))

summary(brafnrasipdBRAF.os)

brafnrasipdBRAF.os <- coxph(Surv(OS, OSstatus)~BRAF+Age+Sex, data=brafnrasipd,
subset=(brafnrasipd$Stage=="I"))

summary(brafnrasipdBRAF.os)

brafnrasipdBRAF.os <- coxph(Surv(OS, OSstatus)~as.numeric(Thickness),
data=brafnrasipd)
summary(brafnrasipdBRAF.os)

brafnrasipdBRAF.os <- coxph(Surv(OS, OSstatus)~BRAF+as.numeric(Thickness),
data=brafnrasipd)
summary(brafnrasipdBRAF.os)
```

```
brafnrasipdBRAF.os <- coxph(Surv(OS,
OSstatus)~BRAF+as.numeric(Thickness)+Stage, data=brafnrasipd)
summary(brafnrasipdBRAF.os)


brafnrasipdBRAF.os <- coxph(Surv(OS, OSstatus)~BRAF+as.numeric(Thickness),
data=brafnrasipd, subset=(brafnrasipd$Stage=="IV"))
summary(brafnrasipdBRAF.os)


brafnrasipdBRAF.os <- coxph(Surv(OS,
OSstatus)~BRAF+as.numeric(Thickness)+Sex+Age, data=brafnrasipd,
subset=(brafnrasipd$Stage=="IV"))
summary(brafnrasipdBRAF.os)


################## Why does BRAF lose its significance in above category?
###############################################################



brafnrasipdBRAF.dfs <- coxph(Surv(DFS, DFSstatus)~BRAF, data=brafnrasipd,
subset=(brafnrasipd$Stage=="I"))

summary(brafnrasipdBRAF.dfs)







# Cox for BRAF and NRAS for stages separately from IPD data
###################################################


brafnrasipdBRAF.dfs <- coxph(Surv(DFS, DFSstatus)~BRAF, data=brafnrasipd,
subset=(brafnrasipd$Stage=="IV"))

summary(brafnrasipdBRAF.dfs)
```

```
brafnrasipdBRAF.os <- coxph(Surv(OS, OSstatus)~BRAF, data=brafnrasipd,
subset=(brafnrasipd$Stage=="IV"))

summary(brafnrasipdBRAF.os)

brafnrasipdBRAF.mss <- coxph(Surv(MSS, MSSstatus)~BRAF, data=brafnrasipd,
subset=(brafnrasipd$Stage=="IV"))

summary(brafnrasipdBRAF.mss)


brafnrasipdNRAS.dfs <- coxph(Surv(DFS, DFSstatus)~NRAS, data=brafnrasipd,
subset=(brafnrasipd$Stage=="IV"))

summary(brafnrasipdNRAS.dfs)

brafnrasipdNRAS.os <- coxph(Surv(OS, OSstatus)~NRAS, data=brafnrasipd,
subset=(brafnrasipd$Stage=="IV"))

summary(brafnrasipdNRAS.os)

brafnrasipdNRAS.mss <- coxph(Surv(MSS, MSSstatus)~NRAS, data=brafnrasipd,
subset=(brafnrasipd$Stage=="IV"))

summary(brafnrasipdNRAS.mss)




brafnrasipdBRAF.dfs <- coxph(Surv(DFS, DFSstatus)~BRAF, data=brafnrasipd,
subset=(brafnrasipd$Stage=="III"))

summary(brafnrasipdBRAF.dfs)

brafnrasipdBRAF.os <- coxph(Surv(OS, OSstatus)~BRAF, data=brafnrasipd,
subset=(brafnrasipd$Stage=="III"))

summary(brafnrasipdBRAF.os)
```

```
brafnrasipdBRAF.mss <- coxph(Surv(MSS, MSSstatus)~BRAF, data=brafnrasipd,
subset=(brafnrasipd$Stage=="III"))

summary(brafnrasipdBRAF.mss)


brafnrasipdNRAS.dfs <- coxph(Surv(DFS, DFSstatus)~NRAS, data=brafnrasipd,
subset=(brafnrasipd$Stage=="III"))

summary(brafnrasipdNRAS.dfs)

brafnrasipdNRAS.os <- coxph(Surv(OS, OSstatus)~NRAS, data=brafnrasipd,
subset=(brafnrasipd$Stage=="III"))

summary(brafnrasipdNRAS.os)

brafnrasipdNRAS.mss <- coxph(Surv(MSS, MSSstatus)~NRAS, data=brafnrasipd,
subset=(brafnrasipd$Stage=="III"))

summary(brafnrasipdNRAS.mss)




brafnrasipdBRAF.dfs <- coxph(Surv(DFS, DFSstatus)~BRAF, data=brafnrasipd,
subset=(brafnrasipd$Stage=="II"))

summary(brafnrasipdBRAF.dfs)

brafnrasipdBRAF.os <- coxph(Surv(OS, OSstatus)~BRAF, data=brafnrasipd,
subset=(brafnrasipd$Stage=="II"))

summary(brafnrasipdBRAF.os)

brafnrasipdBRAF.mss <- coxph(Surv(MSS, MSSstatus)~BRAF, data=brafnrasipd,
subset=(brafnrasipd$Stage=="II"))

summary(brafnrasipdBRAF.mss)
```

```
brafnrasipdNRAS.dfs <- coxph(Surv(DFS, DFSstatus)~NRAS, data=brafnrasipd,
subset=(brafnrasipd$Stage=="II"))

summary(brafnrasipdNRAS.dfs)

brafnrasipdNRAS.os <- coxph(Surv(OS, OSstatus)~NRAS, data=brafnrasipd,
subset=(brafnrasipd$Stage=="II"))

summary(brafnrasipdNRAS.os)

brafnrasipdNRAS.mss <- coxph(Surv(MSS, MSSstatus)~NRAS, data=brafnrasipd,
subset=(brafnrasipd$Stage=="II"))

summary(brafnrasipdNRAS.mss)





##############################################################################
#### Subset per time_from ####################################################

# ### BRAF OS ###


path<-"Z:\\DATA\\"

library("metafor")

library("meta")

melanoma.metaos<-"Final_Aggregate_braf_os.csv"

brafos<-read.csv(paste(path,melanoma.metaos,sep=""),header=TRUE)

brafos.meta<- metagen(TE=brafos$ln_hr_os_braf, seTE=brafos$se_ln_hr_os_braf,
studlab=paste(brafos$author, brafos$Year, sep=", "), sm="HR", level=0.95,
level.comb=0.95, comb.fixed=TRUE, comb.random=TRUE, hakn=FALSE,
method.tau="DL", tau.preset=NULL, TE.tau=NULL, method.bias="linreg",
n.e=brafos$braf_n, n.c=brafos$braf_n_wt, title="HR_OS for BRAF", complab="",
```

```
outclab="", label.e="BRAF", label.c="WT", label.left="", label.right="",
warn=TRUE)

print(brafos.meta, sortvar=brafos$Year, level=brafos.meta$level,
level.comb=brafos.meta$level.comb, comb.fixed=brafos.meta$comb.fixed,
comb.random=brafos.meta$comb.random, details=TRUE, ma=TRUE, digits=2)

win.metafile(filename = "Forest_brafos_timefrom.wmf", width = 10, height = 6.5,
pointsize = 12, restoreConsole = FALSE)
forest(brafos.meta, smlab="HR OS BRAF", sortvar=brafos$Year,
byvar=brafos$time_from)
dev.off()

# metabias(brafos.meta, method.bias="rank", plotit=TRUE, correct=TRUE, k.min=3)
metabias(brafos.meta, method.bias="linreg", plotit=TRUE, correct=FALSE,
k.min=3)
# metabias(brafos.meta, method.bias="mm", plotit=TRUE, correct=FALSE, k.min=3)

# metabias(brafos.meta, method.bias="count", plotit=FALSE, correct=TRUE,
k.min=3)
# method.bias 'count' only defined for meta-analysis with binary outcome data
(function 'metabin')
# metabias(brafos.meta, method.bias="score", plotit=TRUE, correct=FALSE,
k.min=3)
# method.bias 'score' only defined for meta-analysis with binary outcome data
(function 'metabin')
# metabias(brafos.meta, method.bias="peters", plotit=FALSE, correct=FALSE,
k.min=3)
# method.bias 'peters' only defined for meta-analysis with binary outcome data
(function 'metabin')

metainf(brafos.meta, pooled="random", sortvar=brafos$Year,
level.comb=brafos.meta$level.comb)

metacum(brafos.meta, pooled="random", sortvar=brafos$Year,
level.comb=brafos.meta$level.comb)

win.metafile(filename = "Funnel_brafos_timefrom.wmf", width = 12, height = 9,
pointsize = 12, restoreConsole = FALSE)
funnel(brafos.meta, comb.fixed=FALSE, comb.random=TRUE, level=0.95,
contour=c(0.9, 0.95, 0.99), col.contour=c("white", "grey", "lightgrey"), lwd=2,
cex=2, pch=16, studlab=TRUE, cex.studlab=0.75)
```

```
dev.off()

# win.metafile(filename = "Radial_brafos_timefrom.wmf", width = 9, height = 5,
pointsize = 12, restoreConsole = FALSE)
# radial(brafos.meta, comb.fixed=TRUE, axes=TRUE, cex=1, pch=16, level=0.95)
# dev.off()

brafos.trim<-trimfill(brafos.meta)

print(brafos.trim)

win.metafile(filename = "Funnel_brafos.trim_timefrom.wmf", width = 12, height =
9, pointsize = 12, restoreConsole = FALSE)
funnel(brafos.trim, comb.fixed=FALSE, comb.random=TRUE, level=0.95,
contour=c(0.9, 0.95, 0.99), col.contour=c("white", "grey", "lightgrey"), lwd=2,
cex=2, pch=16, studlab=TRUE, cex.studlab=0.75)
dev.off()




win.metafile(filename = "Forest_brafos_timefrom.wmf", width = 10, height = 6.5,
pointsize = 12, restoreConsole = FALSE)
forest(brafos.meta, smlab="HR OS BRAF", sortvar=brafos$Year,
byvar=brafos$time_from)
dev.off()

win.metafile(filename = "Forest_brafos_Continent.wmf", width = 10, height =
10.5, pointsize = 12, restoreConsole = FALSE)
forest(brafos.meta, smlab="HR OS BRAF", sortvar=brafos$Year,
byvar=brafos$Continent)
dev.off()


win.metafile(filename = "Forest_brafos_data_from.wmf", width = 10, height = 8,
pointsize = 12, restoreConsole = FALSE)
forest(brafos.meta, smlab="HR OS BRAF", sortvar=brafos$Year,
byvar=brafos$data_from)
dev.off()
```

```
win.metafile(filename = "Forest_brafos_only_met_patients.wmf", width = 10,
height = 6.5, pointsize = 12, restoreConsole = FALSE)
forest(brafos.meta, smlab="HR OS BRAF", sortvar=brafos$Year,
byvar=brafos$only_met_patients)
dev.off()


win.metafile(filename = "Forest_brafos_Specimen_Type.wmf", width = 10, height =
8, pointsize = 12, restoreConsole = FALSE)
forest(brafos.meta, smlab="HR OS BRAF", sortvar=brafos$Year,
byvar=brafos$Specimen_Type)
dev.off()


win.metafile(filename = "Forest_brafos_design.wmf", width = 10, height = 6.5,
pointsize = 12, restoreConsole = FALSE)
forest(brafos.meta, smlab="HR OS BRAF", sortvar=brafos$Year,
byvar=brafos$design)
dev.off()


win.metafile(filename = "Forest_brafos_braf_exons_2.wmf", width = 10, height =
8, pointsize = 12, restoreConsole = FALSE)
forest(brafos.meta, smlab="HR OS BRAF", sortvar=brafos$Year,
byvar=brafos$braf_exons_2)
dev.off()


win.metafile(filename = "Forest_brafos_IOR.wmf", width = 10, height = 6.5,
pointsize = 12, restoreConsole = FALSE)
forest(brafos.meta, smlab="HR OS BRAF", sortvar=brafos$Year, byvar=brafos$IOR)
dev.off()


win.metafile(filename = "Forest_brafos_SOR.wmf", width = 10, height = 8,
pointsize = 12, restoreConsole = FALSE)
forest(brafos.meta, smlab="HR OS BRAF", sortvar=brafos$Year, byvar=brafos$SOR)
dev.off()


win.metafile(filename = "Forest_brafos_other_risk.wmf", width = 10, height =
6.5, pointsize = 12, restoreConsole = FALSE)
```

```
forest(brafos.meta, smlab="HR OS BRAF", sortvar=brafos$Year,
byvar=brafos$other_risk)
dev.off()



############################ Subgroup analysis for BRAF OSMSS
################################################################################
##############################


path<-"Z:\\DATA\\"

sink("Melanoma_Meta_day 2.txt", append=FALSE, split=TRUE)

library("metafor")

library("meta")

melanoma.metaosmss<-"Final_Aggregate_braf_osmss.csv"

brafosmss<-read.csv(paste(path,melanoma.metaosmss,sep=""),header=TRUE)

brafosmss.meta<- metagen(TE=brafosmss$ln_hr_osmss_braf,
seTE=brafosmss$se_ln_hr_osmss_braf, studlab=paste(brafosmss$author,
brafosmss$Year, sep=", "), sm="HR", level=0.95, level.comb=0.95,
comb.fixed=TRUE, comb.random=TRUE, hakn=FALSE, method.tau="DL",
tau.preset=NULL, TE.tau=NULL, method.bias="linreg", n.e=brafosmss$braf_n,
n.c=brafosmss$braf_n_wt, title="HR_osmss for BRAF", complab="", outclab="",
label.e="BRAF", label.c="WT", label.left="", label.right="", warn=TRUE)

print(brafosmss.meta, sortvar=brafosmss$Year, level=brafosmss.meta$level,
level.comb=brafosmss.meta$level.comb, comb.fixed=brafosmss.meta$comb.fixed,
comb.random=brafosmss.meta$comb.random, details=TRUE, ma=TRUE, digits=2)



######time_from #######################
```

```
win.metafile(filename = "Forest_brafosmss_timefrom.wmf", width = 10, height =
6.5, pointsize = 12, restoreConsole = FALSE)
forest(brafosmss.meta, smlab="HR OS BRAF", sortvar=brafosmss$Year,
byvar=brafosmss$time_from)
dev.off()

melanoma.metaosmssmet<-"Final_Aggregate_braf_osmss_met.csv"

brafosmssmet<-read.csv(paste(path,melanoma.metaosmssmet,sep=""),header=TRUE)

brafosmssmet.meta<- metagen(TE=brafosmssmet$ln_hr_osmss_braf,
seTE=brafosmssmet$se_ln_hr_osmss_braf, studlab=paste(brafosmssmet$author,
brafosmssmet$Year, sep=", "), sm="HR", level=0.95, level.comb=0.95,
comb.fixed=TRUE, comb.random=TRUE, hakn=FALSE, method.tau="DL",
tau.preset=NULL, TE.tau=NULL, method.bias="linreg", n.e=brafosmssmet$braf_n,
n.c=brafosmssmet$braf_n_wt, title="HR_osmss for BRAF", complab="", outclab="",
label.e="BRAF", label.c="WT", label.left="", label.right="", warn=TRUE)

print(brafosmssmet.meta, sortvar=brafosmssmet$Year,
level=brafosmssmet.meta$level, level.comb=brafosmssmet.meta$level.comb,
comb.fixed=brafosmssmet.meta$comb.fixed,
comb.random=brafosmssmet.meta$comb.random, details=TRUE, ma=TRUE, digits=2)




win.metafile(filename = "Forest_brafosmss_Specimen_Type.wmf", width = 11,
height = 9, pointsize = 12, restoreConsole = FALSE)
forest(brafosmss.meta, smlab="HR OS BRAF", sortvar=brafosmss$Year,
byvar=brafosmss$Specimen_Type)
dev.off()

win.metafile(filename = "Forest_brafosmss_data_from.wmf", width = 10, height =
8, pointsize = 12, restoreConsole = FALSE)
forest(brafosmss.meta, smlab="HR OS BRAF", sortvar=brafosmss$Year,
byvar=brafosmss$data_from)
dev.off()

win.metafile(filename = "Forest_brafosmss_Continent.wmf", width = 10, height =
10.5, pointsize = 12, restoreConsole = FALSE)
```

```
forest(brafosmss.meta, smlab="HR OS BRAF", sortvar=brafosmss$Year,
byvar=brafosmss$Continent)
dev.off()

win.metafile(filename = "Forest_brafosmss_design.wmf", width = 10, height =
6.5, pointsize = 12, restoreConsole = FALSE)
forest(brafosmss.meta, smlab="HR OS BRAF", sortvar=brafosmss$Year,
byvar=brafosmss$design)
dev.off()

win.metafile(filename = "Forest_brafosmss_braf_exons_2.wmf", width = 10, height
= 8, pointsize = 12, restoreConsole = FALSE)
forest(brafosmss.meta, smlab="HR OS BRAF", sortvar=brafosmss$Year,
byvar=brafosmss$braf_exons_2)
dev.off()

win.metafile(filename = "Forest_brafosmss_IOR.wmf", width = 10, height = 6.5,
pointsize = 12, restoreConsole = FALSE)
forest(brafosmss.meta, smlab="HR OS BRAF", sortvar=brafosmss$Year,
byvar=brafosmss$IOR)
dev.off()

win.metafile(filename = "Forest_brafosmss_SOR.wmf", width = 10, height = 8,
pointsize = 12, restoreConsole = FALSE)
forest(brafosmss.meta, smlab="HR OS BRAF", sortvar=brafosmss$Year,
byvar=brafosmss$SOR)
dev.off()

win.metafile(filename = "Forest_brafosmss_other_risk.wmf", width = 10, height =
6.5, pointsize = 12, restoreConsole = FALSE)
forest(brafosmss.meta, smlab="HR OS BRAF", sortvar=brafosmss$Year,
byvar=brafosmss$other_risk)
dev.off()



###############################################################################
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############################## Meta-analysis of the IPD studies for OS
###############################################################################
######################

# ### BRAF OS ###

path<-"Z:\\DATA\\"

library("metafor")

library("meta")

melanoma.metaipd<-"IPD_studies_noDeich_noDemu_noDF.csv"

brafosipd<-read.csv(paste(path,melanoma.metaipd,sep=""),header=TRUE)

brafosipd.meta<- metagen(TE=brafosipd$ln_hr_os_braf,
seTE=brafosipd$se_ln_hr_os_braf, studlab=paste(brafosipd$author,
brafosipd$Year, sep=", "), sm="HR", level=0.95, level.comb=0.95,
comb.fixed=TRUE, comb.random=TRUE, hakn=FALSE, method.tau="DL",
tau.preset=NULL, TE.tau=NULL, method.bias="linreg", n.e=brafosipd$braf_n,
n.c=brafosipd$braf_n_wt, title="HR_OS for BRAF", complab="", outclab="",
label.e="BRAF", label.c="WT", label.left="", label.right="", warn=TRUE)

print(brafosipd.meta, sortvar=brafosipd$Year, level=brafosipd.meta$level,
level.comb=brafosipd.meta$level.comb, comb.fixed=brafosipd.meta$comb.fixed,
comb.random=brafosipd.meta$comb.random, details=TRUE, ma=TRUE, digits=2)

win.metafile(filename = "Forest_brafosipd.wmf", width = 10, height = 4.5,
pointsize = 12, restoreConsole = FALSE)
forest(brafosipd.meta, smlab="HR OS BRAF", sortvar=brafosipd$Year)
dev.off()

metabias(brafosipd.meta, method.bias="linreg", plotit=TRUE, correct=FALSE,
k.min=3)

metainf(brafosipd.meta, pooled="random", sortvar=brafosipd$Year,
level.comb=brafosipd.meta$level.comb)
```

```
metacum(brafosipd.meta, pooled="random", sortvar=brafosipd$Year,
level.comb=brafosipd.meta$level.comb)

win.metafile(filename = "Funnel_brafosipd.wmf", width = 12, height = 9,
pointsize = 12, restoreConsole = FALSE)
funnel(brafosipd.meta, comb.fixed=FALSE, comb.random=TRUE, level=0.95,
contour=c(0.9, 0.95, 0.99), col.contour=c("white", "grey", "lightgrey"), lwd=2,
cex=2, pch=16, studlab=TRUE, cex.studlab=0.75)
dev.off()

brafosipd.trim<-trimfill(brafosipd.meta)

print(brafosipd.trim)

win.metafile(filename = "Funnel_brafosipd.trim.wmf", width = 12, height = 9,
pointsize = 12, restoreConsole = FALSE)
funnel(brafosipd.trim, comb.fixed=FALSE, comb.random=TRUE, level=0.95,
contour=c(0.9, 0.95, 0.99), col.contour=c("white", "grey", "lightgrey"), lwd=2,
cex=2, pch=16, studlab=TRUE, cex.studlab=0.75)
dev.off()
```

```
path<-"Z:\\DATA\\"

library("metafor")

melanoma.metaipd<-"IPD_studies_noDeich_noDemu_noDF.csv"

brafosipd<-read.csv(paste(path,melanoma.metaipd,sep=""),header=TRUE)

brafosipd.meta<-rma.uni(yi=ln_hr_os_braf, sei=se_ln_hr_os_braf, data=brafosipd,
measure="GEN", slab=paste(brafosipd$author, brafosipd$Year, sep=", "),
method="DL", knha=FALSE, level=95)

regtest.rma(brafosipd.meta, model="rma", predictor="ninv",
ni=brafosipd$braf_n_total)

win.metafile(filename = "Radial_brafosipd_2.wmf", width = 6, height = 5,
pointsize = 12, restoreConsole = FALSE)
```

```
radial(brafosipd.meta, transf=exp, xlab="Inverse of Standard Error",
zlab="Standardised Treatment Effect (z-score)")
dev.off()
```