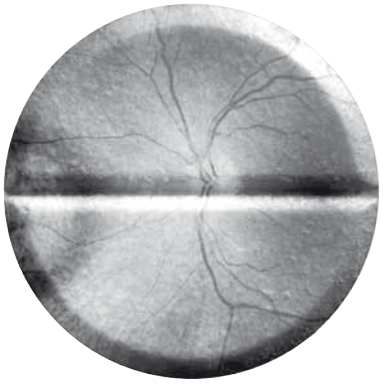
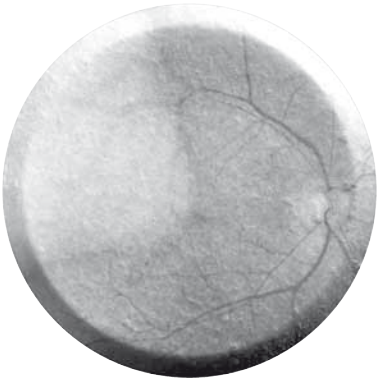
CHAPTER 1

GENERAL INTRODUCTION





AIMS OF THIS THESIS

This thesis describes studies that all strive towards 'Knowledge-based treatment of uveal melanoma' and involves three different yet related topics.

The first part of this thesis analyzes the role of the tumor microenvironment and of malignant development of blood vessels in uveal melanoma and potential treatments.

The second part explores future treatment options in uveal melanoma by development and identification of specific targets for treatment.

The third part focuses on the evaluation of potential cancer treatments and explores different molecular mechanisms that play a role in uveal melanoma growth and dissemination.

UVEAL MELANOMA

Ocular melanoma accounts for only 5% of all melanomas and arises in four ocular tissues, namely the uveal tract, conjunctiva, eyelid, and orbit. Uveal melanoma (UM) is the most common of the ocular melanomas (85 %) with an annual incidence of seven cases per million adults per year in Caucasian populations, and develops in the uvea which consist of the iris, ciliary body and choroid. Most melanocytic tumors of the uveal tract arise in the choroid (80 %), which is one of the most capillary-rich tissues of the body [1]. Symptoms of a uveal melanoma include blurred vision, flashing lights and seeing shadows. Often, there are no symptoms, and therefore 30 % of the tumors is only discovered during routine examination [2].

Although much progress has been made in the last decades in the local treatment of uveal melanoma, prognosis is still poor. Uveal melanoma often is a deadly disease, with a 5-year mortality ranging from 26% to 32% [3]. Uveal melanoma spreads hematogeneously, mainly to the liver [4]. In case of metastases, life expectancy is merely a few months (2-6 months) [5].

Mechanisms involved in uveal melanoma development are largely unknown. Uveal melanoma is approximately 20 to 30 times more common in white people compared to black and Asian people [6]. In addition, light skin color and light iris color are established risk factors [7,8]. Still, unlike cutaneous sun-related malignancies, ultraviolet light exposure does not appear to be a consistent riskfactor in uveal melanoma [9]

Other known risk factors related to survival are histologic cell type, tumor diameter, tumor location, age, and gender [10]. Loss of chromosome 3 is one of the most significant predictors for uveal melanoma-related death [11,12]. In addition, concurrent loss of chromosome 1p and 6q and additional copies of 8q and 6p have also been shown to be potentially implicated in uveal melanoma survival [13,14]. Furthermore, a gene expression-based molecular classification has been described [15,16]. Tumors with a class 2 molecular "signature" are at high risk for the development of metastatic disease compared to tumors with a class 1 "signature".

TREATMENT OF METASTATIC AND PRIMARY UVEAL MELANOMA

Regarding metastatic uveal melanoma, current available treatments include chemotherapy, intra-hepatic arterial liver perfusion, chemoembolization and local resection [17-19]. Although some individual successes have been described using these treatments, unfortunately, the overall survival can as yet only be prolonged with a few months [20].

In case of primary UM, several treatment options are available. For tumors with a prominence of more than 10 mm (or 8.0 mm near the disc), enucleation is often the first choice of treatment. Small to medium-sized tumors with a prominence of less than 10 mm can be treated with proton beam therapy, stereotactic irradiation, or (plaque) brachytherapy, occasionally in combination with transpupillary thermo therapy (sandwich therapy) [21-23,23-25]. Radiation therapy results in destruction of the intraocular tumor, either through direct damage to a cell's DNA, which blocks cell division, or through damage to capillary endothelium, affecting the tumor's blood supply [26-28]. Brachytherapy is a highly successful treatment, achieving local tumor control of UM in 90-97% of the cases [29,30]. Unfortunately, radiation therapy is also associated with a range of complications, including hemorrhages into the tumor or the vitreous, cataract, optic neuropathy, and most commonly, radiation retinopathy.

RADIATION RETINOPATHY

Radiation retinopathy is a slowly-progressive, delayed-onset disease of retinal blood vessels due to changes in the structure and permeability of retinal vessels [31-33], and has been described to occur in up to 63% of eyes after plaque radiation treatment [34-37]. On average, the onset of radiation retinopathy occurs at 26 months after radioactive plaque therapy [38].

Ophthalmoscopic and fluorescein-angiographic findings characteristic of radiation retinopathy include macular changes such as macular edema, capillary non-perfusion, cotton wool spots, capillary telangiectasia, retinal neovascularization, micro aneurysms, retinal hemorrhages, intraretinal exudation, and neuronal changes: disc edema, disc pallor, optic atrophy, and neovascularization of the disk [31] (Figure 1).

Clinically, these signs are often identical to the findings seen in diabetic retinopathy. The threshold for radiation retinopathy depends on the total dose, the volume of irradiated retina, the fractionation scheme, and individual factors. In general, a greater total dose results in earlier, more severe and more pronounced radiation retinopathy [39]. In spite of radiation dose reduction during the last decade, vision loss after therapy remains extensive, particularly vision loss due to macular edema. In one follow-up study, out of 31 uveal melanoma patients with a moderate to good vision ($\geq$ 20/60 or 0.3) before radiation treatment, 5 years later, only 12 showed preservation of vision [21].

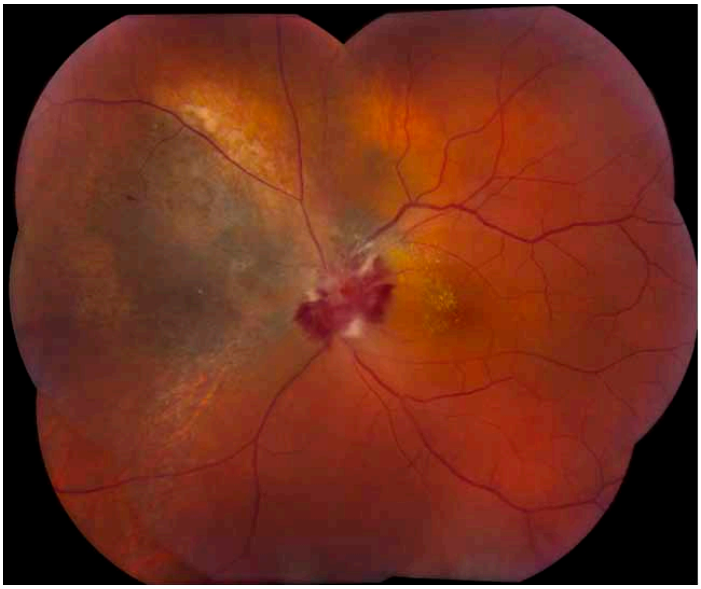


FIGURE 1. Radiation retinopathy due to Ruthenium brachytherapy of a choroidal melanoma.

ANGIOGENESIS AND ANTI-ANGIOGENIC THERAPY

Retinal ischemia and leaking vessels are the two most relevant factors in radiation retinopathy. It has been observed that 55% of eyes, that underwent enucleation after plaque radiation for treatment failure, demonstrate vascular anomalies [34]. As yet, there is no effective treatment to reverse visual loss from ischemic maculopathy due to capillary nonperfusion [31]. However, several anti-angiogenic agents have been used to resolve macular edema and prevent neovascularization. Corticosteroids such as triamcinolone acetonide (TA) have been shown to improve vision for a few months in patients with macular edema associated with diabetic retinopathy [40-43] and in patients with exudative age-related macular degeneration (ARMD) [44]. In addition, a recent study reported a temporary positive effect of intravitreal TA injections in a group of 31 patients with radiation maculopathy [45]. Vascular endothelial growth factor (VEGF) is a pro-angiogenic factor that has a crucial role in the formation of new vessels and most likely contributes to the pathogenesis of radiation retinopathy. Hence anti-angiogenic treatments that target VEGF, such as bevacizumab and ranibizumab, are also used for radiation retinopathy. Anti-angiogenic treatments are further discussed in (*Chapter 3*)

Angiogenesis is an important process in both radiation retinopathy and tumor progression. Besides the supply of oxygen and nutrients, tumor-associated vessels promote metastasis by facilitating tumor cell entry into the circulation [46]. Unquestionably, this phenomenon plays a role in uveal melanoma, which metastasizes almost completely hematogenously [4,5]. Several studies have shown that in uveal melanoma a high vascular density and ingrowth of tumor

cells into the lumen of tumor blood vessels or into a scleral vessel is associated with poor survival [47-49]. Furthermore, uveal melanoma cells are capable of forming a second microcirculation consisting of "looping" patterns of extracellular matrix independent of angiogenesis, called vasculogenic mimicry [50]. The potential to target tumor vessels has been investigated for these past decades in several malignancies and mainly focuses on the key mediator of angiogenesis, which is VEGF. Tumor angiogenesis, the role of VEGF, and anti-angiogenic therapy in cancer and particularly uveal melanoma is further reviewed in *Chapter 3*.

TARGETED THERAPY

Cancer growth depends on many different mechanisms, such as cell proliferation, angiogenesis etc. as described earlier. The combination of different treatments will probably render tumor therapy successful in the future. However, two main obstacles have to be overcome; a specific and effective drug-delivery, and early detection of (micro) metastases. In uveal melanoma, the general accepted hypothesis is that the tumor remains dormant in the bone marrow and liver until becoming clinically detectable in the liver [51]. Identification and inhibition of such micrometastases will prevent the occurrence of 'full-blown' metastatic disease and possibly improve survival in uveal melanoma patients. Effective drug-delivery that induces apoptosis and targets angiogenesis and cell signalling through uveal melanoma-specific ligands would be beneficial.

Other approaches may be immunological. Exploration of (cutaneous) melanoma-derived autoantigens and cell surface receptors has revealed potential targets in uveal melanoma. Currently, the most specific uveal melanoma markers include S-100 (specific for a protein derived from bovine brain cross-reacting with melanoma and melanocytes), HBM45 (specific for gp100) and A103, (recognizes the Melan-A/Mart-1 protein) [52-54]. Sadly, most of these receptors do not exhibit specificity for uveal melanoma and are also expressed by melanocytes or possibly other neural crest-derived cells, and treatment could cause significant side-effects.

PROLIFERATION PATHWAY

What do we know regarding the development and growth of a primary uveal melanoma? Although cutaneous melanoma and uveal melanoma share the same embryonic origin, no similarities can be found regarding mutations that regulate proliferation and cause loss of cell cycle control. Activation of the MAPK pathway in cutaneous melanoma occurs for instance through mutations of the NRAS and BRAF genes (60% of cases) [55,56]. Only BRAF

mutations have been demonstrated in uveal melanoma, though relatively uncommon and are therefore less relevant in this disease [57].

Recently, mutations in uveal melanoma tissue were identified in the GNAQ and GNA11 genes which are located on chromosome 9 and 19, respectively. GNAQ and GNA11 encode for Gαq-type subunits of the heterotrimeric G-protein. Mutations of glutamine encoding codon 209 and (less frequently) R183 can result in constitutive G-protein activation which mediates intracellular signals and activates the RAS-RAF-MEK-ERK or the classical mitogen-activated protein kinase (MAPK) pathway [58]. MAPK activation is crucial in the development of melanocytic neoplasia and constitutive activation of this pathway has been associated with many different types of cancer [59,60]. Mutations in the GNAQ gene were shown to be common in primary UM (45%) and in UM metastases (22%) [61]. GNA11 mutations were present in 32% of primary UM and 57% of UM metastases [62,63]. Cellular components that couple GNAQ/GNA11 to MAPK signaling are most likely the effectors IP3, Src tyrosine kinase [64] and PKC [65].

SRC AND KINASE INHIBITORS

Src is one of the longest known proto-oncogenes and is named after its viral counterpart v-Src, which was first described by Peyton Rous almost 100 years ago [66,67]. Since then, expression and involvement of Src has been demonstrated in several malignancies implicating four 'hallmarks' of cancer; proliferation, angiogenesis, cell survival and migration [66,68,69].

Increased Src signalling correlates with decreased E-cadherin expression and decreased cell-cell adhesion [70,71]. In addition, downstream substrates of Src seem to act largely in parallel to increase cell proliferation and survival [67]. Furthermore, Src activation is associated with increased expression of proangiogenic cytokines such as VEGF, and thus tumor angiogenesis [72,73].

Interestingly, several commercially-available pharmacologic agents are able to inhibit Src. Dasatinib for instance is a small-molecule inhibitor taken orally that has been demonstrated to inhibit proliferation in several malignancies in vitro [74,75] (Figure 2). Like most tyrosine kinase inhibitors, dasatinib also variably inhibits other (receptor) tyrosine kinases such as, BCR-ABL, c-KIT, PDGFR, and ephrin A2 [74,76].

OUTLINE

The first part of this thesis, *Chapter 2 and 3*, describes the literature regarding tumor angiogenesis, the role of vascular endothelial growth factor as key mediator of vessel growth, and the potential of anti-angiogenic therapies that are approved for treatment of several

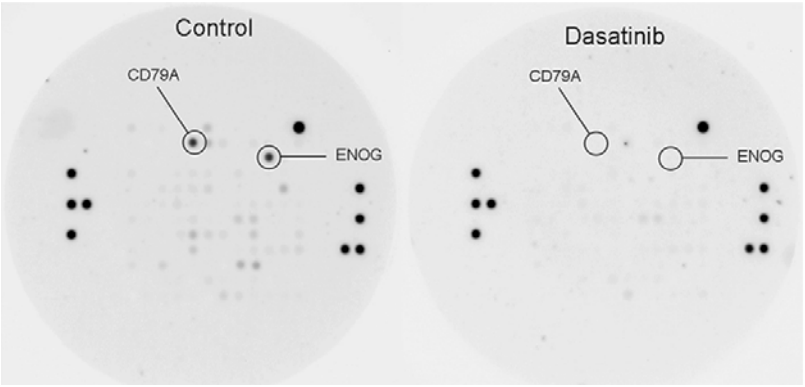


FIGURE 2. Dasatinib treatment (right) inhibits Src kinase-related lysate activity in metastatic uveal tissue analyzed with a Pamgene tyrosine kinase array.

malignancies or may be used in the future. Implications and studies involving uveal melanoma are highlighted in particular. In the second part, *Chapter 4,* tumor angiogenesis in uveal melanoma is experimentally analyzed. We demonstrate the involvement of hypoxia and the HIF-1α pathway in the induction of VEGF expression in uveal melanoma cell lines and cultures. In addition, we describe the association of high VEGF expression in sera of patients and the presence of uveal melanoma related metastases. In *Chapter 5*, we analyze the effect of two frequently used anti-angiogenic intraocular drugs for the treatment of radiation retinopathy caused by irradiation of uveal melanoma. Since local recurrences develop in some cases, the possibility that there are still living uveal melanoma cells present in eyes treated with radiotherapy cannot be excluded [77]. We found that triamcinolone acetonide and anecortave acetate do not stimulate uveal melanoma cell growth in vitro, and therefore most likely will not give rise to recurrences. On the contrary, we demonstrate in *Chapter 6,* that bevacizumab induces intraocular tumor growth in mice. Although bevacizumab did not stimulate UM cell proliferation in vitro, VEGF expression via the HIF-1α pathway was induced, resulting in a 'pseudohypoxic' condition. This phenomenon has been described in other tumor types and may be the consequence of tumor adaptive or evasive resistance.

Part III describes the search for specific uveal melanoma ligands to be used for targeted therapy or early identification of micrometastases. Somatostatin receptor subtype 2 (SSTR2) for instance is expressed in several tumors, and synthetic radiolabelled antagonists like octreotide and octreotate are already being used as diagnostic or therapeutic agents for gastroenteropancreatic and neuroendocrine tumors [78-81]. Although uveal melanoma cells and somatostatin-producing cells both originate from the neural crest, a low expression of SSTR2 was found in primary uveal melanoma specimens and in uveal melanoma cell lines. Furthermore, SSTR2 expression was not associated with tumor-free survival or any known prognostic factor *(Chapter 7).*

On the contrary, we describe in *Chapter 8* the identification of uveal melanoma-specific peptides (UMAPs) by in vitro panning using phage peptide libraries. Additionally, synthetic constructed peptides were shown to successfully internalize targeted UM cells.

In the final chapters of this thesis, the findings of all experiments are summarized and future implications and treatment options are discussed.

Finally, part IV describes the implication of the classical mitogen-activated protein kinase (MAPK) pathway in uveal melanoma. In *Chapter 9*, we identify Src as a determinant of ERK1/2 activation and show that Src expression and kinase activity together with ERK1/2 activation is reduced in UM metastases cell lines compared to primary UM cells. In addition, we demonstrate in *Chapter 10* the inhibition of MAPK activation via Src using the Src kinase inhibitor Dasatinib. Growth arrest was achieved in 60% of the tested UM cultures and the potential of growth inhibition may be predicted by MAPK and Src kinase activity. Furthermore, the sensitive cell cultures predominantly displayed monosomy 3 and, in a large set of UM samples, Src expression level was significantly associated with monosomy 3.

In summary, knowledge of side effects of current intraocular treatments prevents recurrences and may protect the uveal melanoma-containing eye, and hence *preserve vision.*

Pre-clinical evaluation of treatment efficacy and safety based on tumor physiology renders the possibility of new treatment options to treat and/or *prevent metastases.*

REFERENCE LIST

1. Singh, A. D., Bergman, L., and Seregard, S. *Uveal melanoma: epidemiologic aspects.* Ophthalmol.Clin. North Am. 2005. 18:75-84, viii.
2. Damato, B. *Detection of uveal melanoma by optometrists in the United Kingdom.* Ophthalmic Physiol Opt. 2001. 21:268-271.
3. Kujala, E., Makitie, T., and Kivela, T. *Very long-term prognosis of patients with malignant uveal melanoma.* Invest Ophthalmol.Vis.Sci. 2003. 44:4651-4659.
4. Augsburger, J. J., Correa, Z. M., Freire, J., and Brady, L. W. *Long-term survival in choroidal and ciliary body melanoma after enucleation versus plaque radiation therapy.* Ophthalmology 1998. 105:1670-1678.
5. Kujala, E., Makitie, T., and Kivela, T. *Very long-term prognosis of patients with malignant uveal melanoma.* Invest Ophthalmol.Vis.Sci. 2003. 44:4651-4659.
6. Stang, A., Parkin, D. M., Ferlay, J., and Jockel, K. H. *International uveal melanoma incidence trends in view of a decreasing proportion of morphological verification.* Int.J.Cancer 2005. 114:114-123.
7. Gallagher, R. P., Elwood, J. M., Rootman, J., Spinelli, J. J., Hill, G. B., Threlfall, W. J., and Birdsell, J. M. *Risk factors for ocular melanoma: Western Canada Melanoma Study.* J.Natl.Cancer Inst. 1985. 74:775-778.
8. Holly, E. A., Aston, D. A., Char, D. H., Kristiansen, J. J., and Ahn, D. K. *Uveal melanoma in relation to ultraviolet light exposure and host factors.* Cancer Res. 1990. 50:5773-5777.
9. Schmidt-Pokrzywniak, A., Jockel, K. H., Bornfeld, N., Sauerwein, W., and Stang, A. *Positive interaction between light iris color and ultraviolet radiation in relation to the risk of uveal melanoma: a case-control study.* Ophthalmology 2009. 116:340-348.
10. Mooy, C. M. and de Jong, P. T. *Prognostic parameters in uveal melanoma: a review.* Surv.Ophthalmol. 1996. 41:215-228.
11. Prescher, G., Bornfeld, N., Hirche, H., Horsthemke, B., Jockel, K. H., and Becher, R. *Prognostic implications of monosomy 3 in uveal melanoma.* Lancet 1996. 347:1222-1225.
12. Sisley, K., Rennie, I. G., Parsons, M. A., Jacques, R., Hammond, D. W., Bell, S. M., Potter, A. M., and Rees, R. C. *Abnormalities of chromosomes 3 and 8 in posterior uveal melanoma correlate with prognosis.* Genes Chromosomes.Cancer 1997. 19:22-28.
13. Kilic, E., Naus, N. C., van Gils, W., Klaver, C. C., van Til, M. E., Verbiest, M. M., Stijnen, T., Mooy, C. M., Paridaens, D., Beverloo, H. B., Luyten, G. P., and de Klein, A. *Concurrent loss of chromosome arm 1p and chromosome 3 predicts a decreased disease-free survival in uveal melanoma patients.* Invest Ophthalmol.Vis.Sci. 2005. 46:2253-2257.
14. Aalto, Y., Eriksson, L., Seregard, S., Larsson, O., and Knuutila, S. *Concomitant loss of chromosome 3 and whole arm losses and gains of chromosome 1, 6, or 8 in metastasizing primary uveal melanoma.* Invest Ophthalmol.Vis.Sci. 2001. 42:313-317.
15. Onken, M. D., Worley, L. A., Ehlers, J. P., and Harbour, J. W. *Gene expression profiling in uveal melanoma reveals two molecular classes and predicts metastatic death.* Cancer Res. 2004. 64:7205-7209.
16. Tschentscher, F., Husing, J., Holter, T., Kruse, E., Dresen, I. G., Jockel, K. H., Anastassiou, G., Schilling, H., Bornfeld, N., Horsthemke, B., Lohmann, D. R., and Zeschnigk, M. *Tumor classification based on gene expression profiling shows that uveal melanomas with and without monosomy 3 represent two distinct entities.* Cancer Res. 2003. 63:2578-2584.
17. Leyvraz, S., Spataro, V., Bauer, J., Pampallona, S., Salmon, R., Dorval, T., Meuli, R., Gillet, M., Lejeune, F., and Zografos, L. *Treatment of ocular melanoma metastatic to the liver by hepatic arterial chemotherapy.* J.Clin.Oncol. 1997. 15:2589-2595.

18. Mavligit, G. M., Charnsangavej, C., Carrasco, C. H., Patt, Y. Z., Benjamin, R. S., and Wallace, S. *Regression of ocular melanoma metastatic to the liver after hepatic arterial chemoembolization with cisplatin and polyvinyl sponge.* JAMA 1988. 260:974-976.
19. Becker, J. C., Terheyden, P., Kampgen, E., Wagner, S., Neumann, C., Schadendorf, D., Steinmann, A., Wittenberg, G., Lieb, W., and Brocker, E. B. *Treatment of disseminated ocular melanoma with sequential fotemustine, interferon alpha, and interleukin 2.* Br.J.Cancer 2002. 87:840-845.
20. Augsburger, J. J., Correa, Z. M., and Shaikh, A. H. *Effectiveness of treatments for metastatic uveal melanoma.* Am.J.Ophthalmol. 2009. 148:119-127.
21. Bartlema, Y. M., Oosterhuis, J. A., Journee-De Korver, J. G., Tjho-Heslinga, R. E., and Keunen, J. E. *Combined plaque radiotherapy and transpupillary thermotherapy in choroidal melanoma: 5 years' experience.* Br.J.Ophthalmol. 2003. 87:1370-1373.
22. Journee-de Korver, J. G., Oosterhuis, J. A., Kakebeeke-Kemme, H. M., and de Wolff-Rouendaal, D. *Transpupillary thermotherapy (TTT) by infrared irradiation of choroidal melanoma.* Doc.Ophthalmol. 1992. 82:185-191.
23. Melia, B. M., Abramson, D. H., Albert, D. M., Boldt, H. C., Earle, J. D., Hanson, W. F., Montague, P., Moy, C. S., Schachat, A. P., Simpson, E. R., Straatsma, B. R., Vine, A. K., and Weingeist, T. A. *Collaborative ocular melanoma study (COMS) randomized trial of I-125 brachytherapy for medium choroidal melanoma. I. Visual acuity after 3 years COMS report no. 16.* Ophthalmology 2001. 108:348-366.
24. Gragoudas, E. S., Lane, A. M., Munzenrider, J., Egan, K. M., and Li, W. *Long-term risk of local failure after proton therapy for choroidal/ciliary body melanoma.* Trans.Am.Ophthalmol.Soc. 2002. 100:43-48.
25. Gragoudas, E. S., Goitein, M., Verhey, L., Munzenreider, J., Suit, H. D., and Koehler, A. *Proton beam irradiation. An alternative to enucleation for intraocular melanomas.* Ophthalmology 1980. 87:571-581.
26. Gragoudas, E. S., Goitein, M., Verhey, L., Munzenreider, J., Suit, H. D., and Koehler, A. *Proton beam irradiation. An alternative to enucleation for intraocular melanomas.* Ophthalmology 1980. 87:571-581.
27. Char, D. H., Castro, J. R., Quivey, J. M., Phillips, T. L., Irvine, A. R., Stone, R. D., and Kroll, S. *Uveal melanoma radiation. 125I brachytherapy versus helium ion irradiation.* Ophthalmology 1989. 96:1708-1715.
28. Goodman, D. F., Char, D. H., Crawford, J. B., Stone, R. D., and Castro, J. R. *Uveal melanoma necrosis after helium ion therapy.* Am.J.Ophthalmol. 1986. 101:643-645.
29. Shields, C. L., Cater, J., Shields, J. A., Chao, A., Krema, H., Materin, M., and Brady, L. W. *Combined plaque radiotherapy and transpupillary thermotherapy for choroidal melanoma: tumor control and treatment complications in 270 consecutive patients.* Arch.Ophthalmol. 2002. 120:933-940.
30. Jampol, L. M., Moy, C. S., Murray, T. G., Reynolds, S. M., Albert, D. M., Schachat, A. P., Diddie, K. R., Engstrom, R. E., Jr., Finger, P. T., Hovland, K. R., Joffe, L., Olsen, K. R., and Wells, C. G. *The COMS randomized trial of iodine 125 brachytherapy for choroidal melanoma: IV. Local treatment failure and enucleation in the first 5 years after brachytherapy. COMS report no. 19.* Ophthalmology 2002. 109:2197-2206.
31. Guyer, D. R., Mukai, S., Egan, K. M., Seddon, J. M., Walsh, S. M., and Gragoudas, E. S. *Radiation maculopathy after proton beam irradiation for choroidal melanoma.* Ophthalmology 1992. 99:1278-1285.
32. Archer, D. B. and Gardiner, T. A. *Ionizing radiation and the retina.* Curr.Opin.Ophthalmol. 1994. 5:59-65.
33. Archer, D. B., Amoaku, W. M., and Gardiner, T. A. *Radiation retinopathy--clinical, histopathological, ultrastructural and experimental correlations.* Eye (Lond) 1991. 5 (Pt 2):239-251.

34. Sia, S., Harper, C., McAllister, I., and Perry, A. *Iodine-I25 episcleral plaque therapy in uveal melanoma.* Clin.Experiment.Ophthalmol. 2000. 28:409-413.
35. Stack, R., Elder, M., Abdelaal, A., Hidajat, R., and Clemett, R. *New Zealand experience of I125 brachytherapy for choroidal melanoma.* Clin.Experiment.Ophthalmol. 2005. 33:490-494.
36. Jensen, A. W., Petersen, I. A., Kline, R. W., Stafford, S. L., Schomberg, P. J., and Robertson, D. M. *Radiation complications and tumor control after 125I plaque brachytherapy for ocular melanoma.* Int.J.Radiat.Oncol.Biol.Phys. 2005. 63:101-108.
37. Jones, R., Gore, E., Mieler, W., Murray, K., Gillin, M., Albano, K., and Erickson, B. *Posttreatment visual acuity in patients treated with episcleral plaque therapy for choroidal melanomas: dose and dose rate effects.* Int.J.Radiat.Oncol.Biol.Phys. 2002. 52:989-995.
38. Finger, P. T. and Kurli, M. *Laser photocoagulation for radiation retinopathy after ophthalmic plaque radiation therapy.* Br.J.Ophthalmol. 2005. 89:730-738.
39. Harris, J. R. and Levene, M. B. *Visual complications following irradiation for pituitary adenomas and craniopharyngiomas.* Radiology 1976. 120:167-171.
40. Ferrara, N., Gerber, H. P., and LeCouter, J. *The biology of VEGF and its receptors.* Nat.Med. 2003. 9:669-676.
41. Er, H. and Yilmaz, H. *Intravitreal cortisone injection for refractory diffuse diabetic macular edema.* Ophthalmologica 2005. 219:394-400.
42. Patelli, F., Fasolino, G., Radice, P., Russo, S., Zumbo, G., DI Tizio, F. M., Frisone, G., and Marchi, S. *Time course of changes in retinal thickness and visual acuity after intravitreal triamcinolone acetonide for diffuse diabetic macular edema with and without previous macular laser treatment.* Retina 2005. 25:840-845.
43. Sorensen, T. L., Haamann, P., Villumsen, J., and Larsen, M. *Intravitreal triamcinolone for macular oedema: efficacy in relation to aetiology.* Acta Ophthalmol.Scand. 2005. 83:67-70.
44. Jonas, J. B., Spandau, U. H., Kamppeter, B. A., and Harder, B. *Follow-up after intravitreal triamcinolone acetonide for exudative age-related macular degeneration.* Eye 2007. 21:387-394.
45. Shields, C. L., Demirci, H., Dai, V., Marr, B. P., Mashayekhi, A., Materin, M. A., Manquez, M. E., and Shields, J. A. *Intravitreal triamcinolone acetonide for radiation maculopathy after plaque radiotherapy for choroidal melanoma.* Retina 2005. 25:868-874.
46. Papetti, M. and Herman, I. M. *Mechanisms of normal and tumor-derived angiogenesis.* Am.J.Physiol Cell Physiol 2002. 282:C947-C970.
47. Makitie, T., Summanen, P., Tarkkanen, A., and Kivela, T. *Microvascular density in predicting survival of patients with choroidal and ciliary body melanoma.* Invest Ophthalmol.Vis.Sci. 1999. 40:2471-2480.
48. Overkleeft, E. N., Zuidervaart, W., Hurks, H. M., Eilers, P. H., Wolff-Rouendaal, D., and Jager, M. J. *Prognostic value of the disodium phosphate 32P uptake test in uveal melanoma: a long-term study.* Arch.Ophthalmol. 2003. 121:1398-1403.
49. Foss, A. J., Alexander, R. A., Jefferies, L. W., Hungerford, J. L., Harris, A. L., and Lightman, S. *Microvessel count predicts survival in uveal melanoma.* Cancer Res. 1996. 56:2900-2903.
50. Folberg, R., Pe'er, J., Gruman, L. M., Woolson, R. F., Jeng, G., Montague, P. R., Moninger, T. O., Yi, H., and Moore, K. C. *The morphologic characteristics of tumor blood vessels as a marker of tumor progression in primary human uveal melanoma: a matched case-control study.* Hum.Pathol. 1992. 23:1298-1305.
51. Eide, N., Faye, R. S., Hoifodt, H. K., Overgaard, R., Jebsen, P., Kvalheim, G., and Fodstad, O. *Immunomagnetic detection of micrometastatic cells in bone marrow in uveal melanoma patients.* Acta Ophthalmol. 2009. 87:830-836.

52. Stefansson, K., Wollmann, R., and Jerkovic, M. *S-100 protein in soft-tissue tumors derived from Schwann cells and melanocytes.* Am.J.Pathol. 1982. 106:261-268.
53. Adema, G. J., de Boer, A. J., van 't, H. R., Denijn, M., Ruiter, D. J., Vogel, A. M., and Figdor, C. G. *Melanocyte lineage-specific antigens recognized by monoclonal antibodies NKI-beteb, HMB-50, and HMB-45 are encoded by a single cDNA.* Am.J.Pathol. 1993. 143:1579-1585.
54. Heegaard, S., Jensen, O. A., and Prause, J. U. *Immunohistochemical diagnosis of malignant melanoma of the conjunctiva and uvea: comparison of the novel antibody against melan-A with S100 protein and HMB-45.* Melanoma Res. 2000. 10:350-354.
55. Davies, H., Bignell, G. R., Cox, C., Stephens, P., Edkins, S., Clegg, S., Teague, J., Woffendin, H., Garnett, M. J., Bottomley, W., Davis, N., Dicks, E., Ewing, R., Floyd, Y., Gray, K., Hall, S., Hawes, R., Hughes, J., Kosmidou, V., Menzies, A., Mould, C., Parker, A., Stevens, C., Watt, S., Hooper, S., Wilson, R., Jayatilake, H., Gusterson, B. A., Cooper, C., Shipley, J., Hargrave, D., Pritchard-Jones, K., Maitland, N., Chenevix-Trench, G., Riggins, G. J., Bigner, D. D., Palmieri, G., Cossu, A., Flanagan, A., Nicholson, A., Ho, J. W., Leung, S. Y., Yuen, S. T., Weber, B. L., Seigler, H. F., Darrow, T. L., Paterson, H., Marais, R., Marshall, C. J., Wooster, R., Stratton, M. R., and Futreal, P. A. *Mutations of the BRAF gene in human cancer.* Nature 2002. 417:949-954.
56. van, E. A., Zerp, S., van der Flier, S., Kruse-Wolters, M., Vacca, A., Ruiter, D. J., and Schrier, P. *Analysis of N-ras mutations in human cutaneous melanoma: tumor heterogeneity detected by polymerase chain reaction/single-stranded conformation polymorphism analysis.* Recent Results Cancer Res. 1995. 139:57-67.
57. Maat, W., Kilic, E., Luyten, G. P., de, K. A., Jager, M. J., Gruis, N. A., and van der Velden, P. A. *Pyrophosphorolysis detects B-RAF mutations in primary uveal melanoma.* Invest Ophthalmol.Vis.Sci. 2008. 49:23-27.
58. Hubbard, K. B. and Hepler, J. R. *Cell signalling diversity of the Gqalpha family of heterotrimeric G proteins.* Cell Signal. 2006. 18:135-150.
59. Goding, C. R. *Mitf from neural crest to melanoma: signal transduction and transcription in the melanocyte lineage.* Genes Dev. 2000. 14:1712-1728.
60. Reddy, K. B., Nabha, S. M., and Atanaskova, N. *Role of MAP kinase in tumor progression and invasion.* Cancer Metastasis Rev. 2003. 22:395-403.
61. Van Raamsdonk, C. D., Bezrookove, V., Green, G., Bauer, J., Gaugler, L., O'Brien, J. M., Simpson, E. M., Barsh, G. S., and Bastian, B. C. *Frequent somatic mutations of GNAQ in uveal melanoma and blue naevi.* Nature. 2008.
62. Van Raamsdonk, C. D., Griewank, K. G., Crosby, M. B., Garrido, M. C., Vemula, S., Wiesner, T., Obenauf, A. C., Wackernagel, W., Green, G., Bouvier, N., Sozen, M. M., Baimukanova, G., Roy, R., Heguy, A., Dolgalev, I., Khanin, R., Busam, K., Speicher, M. R., O'Brien, J., and Bastian, B. C. *Mutations in GNA11 in uveal melanoma.* N.Engl.J.Med. 2010. 363:2191-2199.
63. Fisher, D. E., Barnhill, R., Hodi, F. S., Herlyn, M., Merlino, G., Medrano, E., Bastian, B., Landi, T. M., and Sosman, J. *Melanoma from bench to bedside: meeting report from the 6th international melanoma congress.* Pigment Cell Melanoma Res. 2010. 23:14-26.
64. Cramer, H., Schmenger, K., Heinrich, K., Horstmeyer, A., Boning, H., Breit, A., Piiper, A., Lundstrom, K., Muller-Esterl, W., and Schroeder, C. *Coupling of endothelin receptors to the ERK/MAP kinase pathway. Roles of palmitoylation and G(alpha)q.* Eur.J.Biochem. 2001. 268:5449-5459.
65. Patel, M., Smyth, E., Chapman, P. B., Wolchok, J. D., Schwartz, G. K., Abramson, D. H., and Carvajal, R. D. *Therapeutic implications of the emerging molecular biology of uveal melanoma.* Clin.Cancer Res. 2011. 17:2087-2100.
66. Yeatman, T. J. *A renaissance for SRC.* Nat.Rev.Cancer 2004. 4:470-480.

67. Martin, G. S. *The hunting of the Src.* Nat.Rev.Mol.Cell Biol. 2001. 2:467-475.
68. Hanahan, D. and Weinberg, R. A. *The hallmarks of cancer.* Cell 2000. 100:57-70.
69. Alvarez, R. H., Kantarjian, H. M., and Cortes, J. E. *The role of Src in solid and hematologic malignancies: development of new-generation Src inhibitors.* Cancer 2006. 107:1918-1929.
70. Irby, R. B. and Yeatman, T. J. *Increased Src activity disrupts cadherin/catenin-mediated homotypic adhesion in human colon cancer and transformed rodent cells.* Cancer Res. 2002. 62:2669-2674.
71. Nam, J. S., Ino, Y., Sakamoto, M., and Hirohashi, S. *Src family kinase inhibitor PP2 restores the E-cadherin/catenin cell adhesion system in human cancer cells and reduces cancer metastasis.* Clin. Cancer Res. 2002. 8:2430-2436.
72. Kanda, S., Miyata, Y., Kanetake, H., and Smithgall, T. E. *Non-receptor protein-tyrosine kinases as molecular targets for antiangiogenic therapy (Review).* Int.J.Mol.Med. 2007. 20:113-121.
73. Mukhopadhyay, D., Tsiokas, L., Zhou, X. M., Foster, D., Brugge, J. S., and Sukhatme, V. P. *Hypoxic induction of human vascular endothelial growth factor expression through c-Src activation.* Nature 1995. 375:577-581.
74. Chang, Q., Jorgensen, C., Pawson, T., and Hedley, D. W. *Effects of dasatinib on EphA2 receptor tyrosine kinase activity and downstream signalling in pancreatic cancer.* Br.J.Cancer 2008. 99:1074-1082.
75. Lombardo, L. J., Lee, F. Y., Chen, P., Norris, D., Barrish, J. C., Behnia, K., Castaneda, S., Cornelius, L. A., Das, J., Doweyko, A. M., Fairchild, C., Hunt, J. T., Inigo, I., Johnston, K., Kamath, A., Kan, D., Klei, H., Marathe, P., Pang, S., Peterson, R., Pitt, S., Schieven, G. L., Schmidt, R. J., Tokarski, J., Wen, M. L., Wityak, J., and Borzilleri, R. M. *Discovery of N-(2-chloro-6-methyl- phenyl)-2-(6-(4-(2-hydroxyethyl)-piperazin-1-yl)-2-methylpyrimidin-4- ylamino)thiazole-5-carboxamide (BMS-354825), a dual Src/Abl kinase inhibitor with potent antitumor activity in preclinical assays.* J.Med.Chem. 2004. 47:6658-6661.
76. Harbour, J. W., Char, D. H., Kroll, S., Quivey, J. M., and Castro, J. *Metastatic risk for distinct patterns of postirradiation local recurrence of posterior uveal melanoma.* Ophthalmology 1997. 104:1785-1792.
77. Pe'er, J., Stefani, F. H., Seregard, S., Kivela, T., Lommatzsch, P., Prause, J. U., Sobottka, B., Damato, B., and Chowers, I. *Cell proliferation activity in posterior uveal melanoma after Ru-106 brachytherapy: an EORTC ocular oncology group study.* Br.J.Ophthalmol. 2001. 85:1208-1212.
78. de, J. M., Breeman, W. A., Valkema, R., Bernard, B. F., and Krenning, E. P. *Combination radionuclide therapy using 177Lu- and 90Y-labeled somatostatin analogs.* J.Nucl.Med. 2005. 46 Suppl 1:13S-17S.
79. Krenning, E. P., de, J. M., Kooij, P. P., Breeman, W. A., Bakker, W. H., de Herder, W. W., Van Eijck, C. H., Kwekkeboom, D. J., Jamar, F., Pauwels, S., and Valkema, R. *Radiolabelled somatostatin analogue(s) for peptide receptor scintigraphy and radionuclide therapy.* Ann.Oncol. 1999. 10 Suppl 2:S23-S29.
80. Krenning, E. P., Valkema, R., Kwekkeboom, D. J., de Herder, W. W., Van Eijck, C. H., de, J. M., Pauwels, S., and Reubi, J. C. *Molecular imaging as in vivo molecular pathology for gastroenteropancreatic neuroendocrine tumors: implications for follow-up after therapy.* J.Nucl.Med. 2005. 46 Suppl 1:76S-82S.
81. Kwekkeboom, D. J., Mueller-Brand, J., Paganelli, G., Anthony, L. B., Pauwels, S., Kvols, L. K., O'dorisio, T. M., Valkema, R., Bodei, L., Chinol, M., Maecke, H. R., and Krenning, E. P. *Overview of results of peptide receptor radionuclide therapy with 3 radiolabeled somatostatin analogs.* J.Nucl.Med. 2005. 46 Suppl 1:62S-66S.