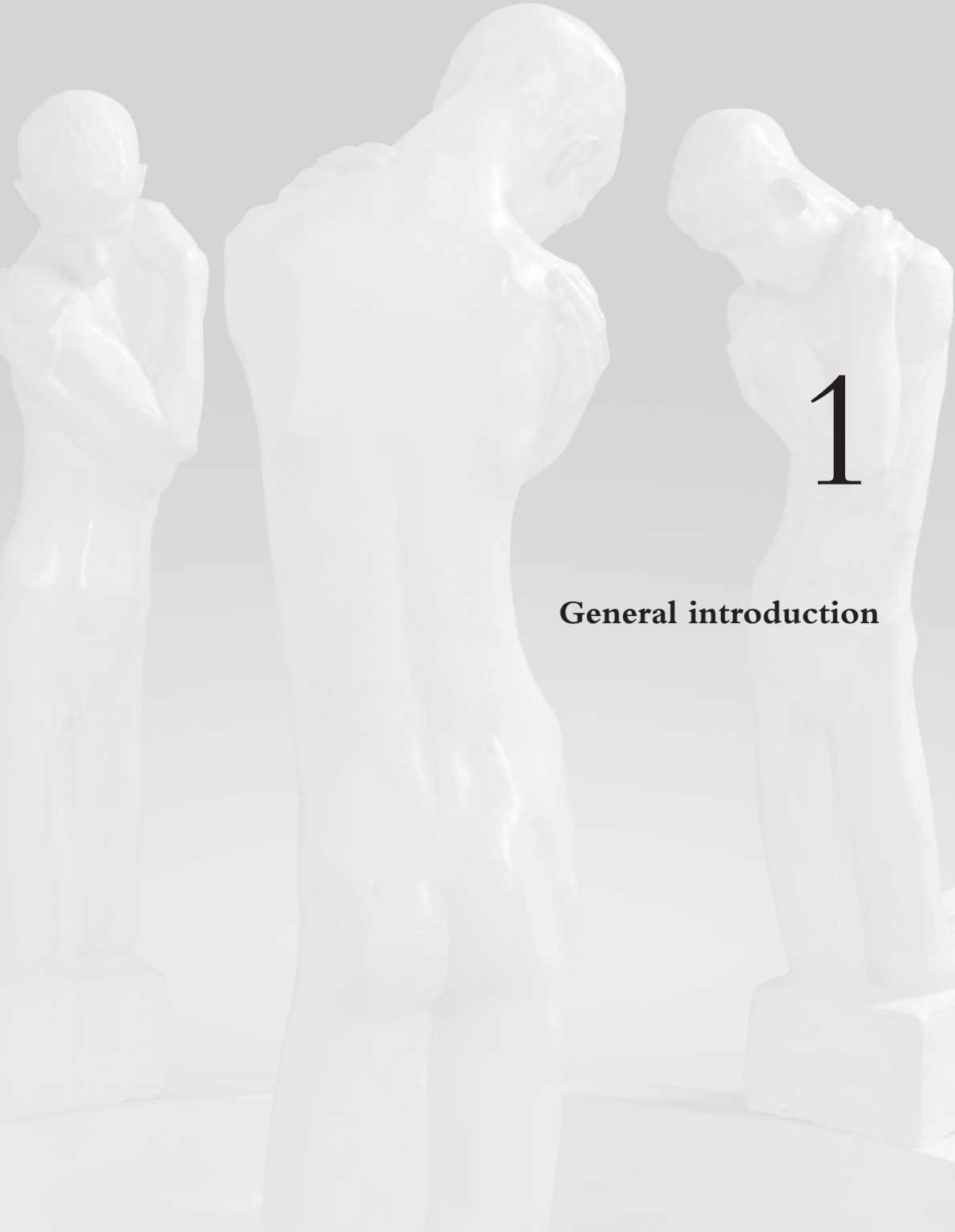

1

General introduction

1

I. Embryology: the vascular system and endothelial cells

The vertebrate circulatory system is formed by two distinct processes during embryogenesis. At first, there is vasculogenesis, defined as de novo blood vessel formation by differentiation of mesoderm-derived endothelial precursor cells [(haem)angioblasts][1, 2]. This is followed by angiogenesis, which includes the proliferation of endothelial cells and formation of new blood vessels from pre-existing vessels[1,2].

The vascular system, consisting of blood vessels and the heart, is the first identifiable structure in the developing embryo[3,4]. The first signs of blood vessel formation occur in the gastrulation stage at day 7.5 in the blood islands of the yolk sac[1,5]. Subsequently, the blood island fuses and forms an immature vascular network, known as the primary plexus. In a next phase, vascular remodelling in the yolk sac leads to the formation of the complex yolk sac vasculature[1,2]. At the same time, angioblasts aggregate into solid endothelial strands along both sides of the neural tube in the embryo[2,5]. They differentiate into the dorsal aorta, vitelline vessels and primary plexuses of lungs, spleen and heart[1]. Although the exact embryogenesis is not fully elucidated, it is clear that these two processes are driven by different signalling pathways and transcriptional regulators. Fibroblast growth factor 2 (FGF2), bone morphogenetic protein 4 (BMP4), its downstream target Indian Hedgehog (IHH), vascular endothelial growth factor A(VEGF-A) and E-twenty six (ETS) transcription factors are key players in vasculogenesis and are important for the differentiation of endothelial and hematopoietic cells[6]. VEGF-A, retinoic acid, transforming growth factor beta (TGF-b), Notch and VEGF signalling pathways are more important during endothelial cell proliferation and angiogenic sprouting, which is also known as angiogenesis[6].

II. Endothelial markers

The starting point of an adequate diagnosis is conventional histological examination using haematoxylin and eosin stained slides, in combination with clinical and/ or radiographical information. In addition, immunohistochemistry is a very useful and sometimes even an essential diagnostic tool to evaluate or confirm the line of differentiation in tumours. Because the classification of vascular tumours can be difficult, different antibodies have been described and used to demonstrate endothelial differentiation. The most commonly used markers are described below.

CD31, also known as platelet endothelial cell adhesion molecule 1 (PECAM-1), is a single-chain type I transmembrane protein member of the immunoglobulin (Ig) superfamily and has a molecular weight of ~83kDa. The extracellular domain contains six Ig-like homology units of the C2 subclass, similar to cell-cell adhesion molecules. CD31 plays a role in angiogenesis[7,8] and diapedesis of leukocytes[9]. It is abundantly expressed on the surface of embryonic vascular and adult endothelial cells[10,11]. Therefore, it has been regarded over the past years as the most sensitive immunohistochemical marker for endothelial cell differentiation. However, it is not an

absolutely specific marker because it is also expressed in macrophages, dendritic cells, platelets, monocytes, neutrophils and a subset of T- en B-lymphocytes and natural killer cells[10,11]. In cultured endothelial cells it is diffusely distributed in the plasma membrane, but when cell-cell contacts are formed it is concentrated at the regions of cell-cell contact[12] where it plays a role in endothelial cell contact[10].

CD34, also known as hematopoietic progenitor cell antigen CD34, is a heavily glycosylated type I transmembrane protein with a molecular weight of ~41kDa. The function of CD34 is still not completely elucidated. To date, it is accepted that CD34 plays a role in cell adhesion and cell transduction[10,13]. CD34 is expressed on the most primitive pluripotential stem cells and hematopoietic progenitor cells of all lineages. The expression is eventually gradually lost when lineage committed progenitors differentiate. It is also present in dendritic interstitial cells, dermal dendrocytes, endometrial stroma, some lymphatic endothelial cells, interstitial cells of Cajal and a subset of fibroblasts[4,10,14]. Because of the expression on endothelial cells and vascular tumours, CD34 is used as a vascular marker. However, compared to CD31, CD34 is less sensitive and less specific[10].

von Willebrand Factor (vWF), also known as Factor VIII-related antigen, is a multimeric plasma glycoprotein with a molecular weight of ~250 kDa. It is synthesized by endothelial cells, megakaryocytes and platelets. In endothelial cells it is located within the Weibel-Palade bodies. vWF has a dual key role in both primary and secondary hemostasis by mediating the adhesion of platelets towards the wound site and chaperoning clotting factor VIII[10, 15]. Mutations of the vWF gene are known to cause von Willebrand disease and are characterized by ecchymoses, hemorrhage and a prolonged bleeding time. vWF is a highly specific endothelial marker. However, it is much less sensitive compared to CD31 and CD34[4,10].

FLI1 (Friend leukaemia integration 1 transcription factor) is a member of the ETS family of DNA binding transcription factors and has a molecular weight of ~51kDa[16]. FLI1 is involved in cellular proliferation and tumourigenesis. It was first discovered in Ewing sarcoma, since approximately 90% of these tumours have a specific translocation, t(11;22)(q24;q12), resulting in the EWSR1-FLI1 fusion protein[17]. However, a study of small blue round cell tumours revealed also nuclear expression of FLI1 in normal endothelial cells and small lymphocytes[16] and therefore FLI1 was reported as the first nuclear marker of endothelial differentiation. However, it is also expressed in a small subset of melanomas, Merkel cell carcinomas, synovial sarcomas, breast carcinomas[18], and nearly all epithelioid sarcomas show positive staining for FLI1[3].

ERG, also known as avian v-ets erytroblastosis virus E26 oncogene homolog, is a member of the ETS family of transcription factors and has a molecular weight of ~55 kDa. It is constitutively expressed by endothelial cells and it has been shown to play a role in the regulation of angiogenesis and apoptosis of endothelial cells[4,10,19]. Recent immunohistochemical studies have demonstrated that ERG is a highly specific endothelial marker. However, it has been shown that

it also plays an important role in carcinogenesis of a subset of prostate carcinomas containing the TMPRSS2-ERG fusion[4,20-22]. Moreover, some myeloid precursor cells[4,23] and rare cases of Ewing sarcomas, containing the EWSR1-ERG fusion, are positive as well[4]. Recent publications have shown that ERG expression is also present in one and up to two third of the epithelioid sarcomas, especially when an antibody directed against the ERG N-terminus was used[3,4].

D2-40 is a commercially available monoclonal antibody directed against human podoplanin. Podoplanin, also known as type I alveolar cell marker hT1alpha-2 and Aggrus, is a type I transmembrane glycoprotein with extensive O-glycosylation and a molecular weight of ~40kDa. Some studies have demonstrated that it is regulated by the lymphatic-specific homeobox gene *Prox 1*, a transcription factor responsible for the development of lymphatic progenitors from embryonic veins[24]. Podoplanin promotes platelet aggregation, and possesses a platelet aggregation-stimulating (PLAG) domain[25]. Podoplanin is expressed in a large number of normal tissue cells, such as mesothelial cells, osteocytes and osteoblasts, follicular dendritic cells of lymphoid tissue, etc…, but also in a variety of different tumour types, in example a subset of vascular tumours, epithelioid mesothelioma, adrenal cortical carcinoma, seminoma/dysgerminoma[26].

Ulex Europaeus Agglutinin 1 (UEA-1) is one of the oldest endothelial markers and has been used for some time. UEA-1 binds to glycoproteins and glycolipids in endothelial cells. However, these glycoproteins and glycolipids are also found in red blood cells and epithelial cells of ABH blood group secretors[27]. Because UEA-1 also reacts with all kinds of epithelial cells, it has no longer any diagnostic value since the discovery of more sensitive and specific endothelial markers such as CD34 and CD31.

Other endothelial markers

Over the years, many other markers such as LYVE-1, Prox1, claudin 5, WT1, VEGF and GLUT1 have been used or are still used as endothelial markers, sometimes to emphasize specific differentiation of the endothelium. However, these markers are not exclusively positive on endothelial cells and therefore not widely used and studied.

III. Vascular tumours

It is generally accepted that vascular tissue or tissue with the immunohistochemical repertoire of endothelial cells as discussed above can give rise to tumours and tumour-like malformations in the skin, soft tissue and viscera. These lesions are part of a wide spectrum of entities, and for clinical purposes classification schemes have been proposed and evolved over the years. Benign haemangiomas are one of the most common soft tissue tumours, whereas angiosarcomas are their malignant counterpart and are extremely rare, highly aggressive and comprise less than 1% of all sarcomas[28]. Other vascular lesions, originally grouped as haemangioendotheliomas, are

more aggressive as compared to benign haemangiomas while they are not full blown malignant angiosarcomas. To overcome problems with ambiguous terms such as "intermediate malignancy" or "borderline malignant potential", the 2002 World Health Organization classification endeavoured to classify soft tissue tumours into four categories: overtly benign lesions, locally aggressive not metastasizing lesions, locally aggressive rarely metastasizing lesions and frankly malignant lesions. In 2013, also vascular tumours of bone were classified as such[29] (Table 1)

Table 1. Overview of all vascular tumours of soft tissue and bone as listed in the 2013 WHO Classification of Tumours of Soft Tissue and Bone.

	Vascular Tumours of Soft Tissue	Vascular Tumours of Bone
Benign	Haemangioma synovial venous arteriovenous haemangioma/ malformation epithelioid haemangioma angiomatosis lymphangioma	Haemangioma
Intermediate (locally aggressive)	Kaposiform haemangioendothelioma	Epithelioid haemangioma
Intermediate (rarely metastasizing)	Retiform haemangioendothelioma Papillary intralymphatic angioendothelioma Composite haemangioendothelioma Kaposi sarcoma Pseudomyogenic (epithelioid sarcoma-like) haemangioendothelioma	
Malignant	Epithelioid haemangioendothelioma Angiosarcoma of soft tissue	Epithelioid haemangioendothelioma Angiosarcoma

IV. Vascular tumours of bone

In bone, the nutrient arteries penetrate the cortex and branch into an abundant network of small arteries and capillaries. The terminology of vascular tumours of bone has been a matter of debate over the years, which has led to the use of different terminology and different classification systems used simultaneously over the years (Table 2). None of these classification systems have been generally accepted due to the lack of consistent terminology, accepted histological criteria and only a limited correlation with clinical outcome. In particular, the classification of vascular tumours of bone belonging to the intermediate category, or representing low-grade vascular tumours of bone, previously simply grouped as "haemangioendotheliomas", has been extremely difficult. Solitary haemangiomas are relatively common in bone. Autopsy studies have reported that haemangiomas are present in up to 10%[30]. These lesions occur most frequent as

an asymptomatic incidental finding in the skull or spine, although extraspinal locations are also reported. However, despite the rich vascularity of bone primary malignant vascular tumours of bone are extremely rare[31]. They represent less than 1% of primary malignant bone tumours reported by the Netherlands Committee on Bone tumours[32] and 0,5% of those registered at the Mayo Clinic[33]. Because of their rareness, little systematic knowledge is available. Clinically they seem to be extremely aggressive and have a very poor prognosis. Currently, epithelioid haemangioma, previously also known as haemangioendothelioma of bone, has been recognized as a locally aggressive vascular neoplasm with distinct histological criteria and an excellent prognosis[29,34]. The term "haemangiopericytoma" of soft tissue was already abandoned in the 2002 World Health Organisation (WHO) Classification of Tumours of Soft Tissue and Bone.
It has been accepted that it merely represents a nonspecific growth pattern exhibited by a large number of tumours, and "haemangiopericytoma of soft tissue" could be reclassified as solitary fibrous tumours, monophasic synovial sarcoma, and (infantile) myofibromatosis or myofibroblastic laesions[29,35,36]. Similar lesions occur in bone, which led us to question whether also "haemangiopericytoma of bone" is a true entity (see Chapter 7). In the 2013 WHO classification "haemangiopericytoma of bone" is also no longer recognized as a separate entity.
The characteristics of the different histological types of vascular lesions of bone are described in more detail in Chapter 2 of this thesis.

V. Multifocality

Both benign and malignant vascular tumours of bone can occur multifocally, and multifocal disease in bone is even more common as compared to the same tumours occurring in soft tissue. The distribution of these lesions can be either contiguous, involving adjacent bones, or non-contiguous, involving two or more distant sites[37]. Multiple benign vascular lesions, known as haemangiomatosis, are considered a developmental disorder (vascular hamartomas)[37]. Up to one third of the angiosarcoma of bone and nearly two third of the epithelioid haemangioendotheliomas of bone occur multifocally[29]. Although the exact mechanism remains unclear, one could speculate i) they could be metastatic lesions (skip metastasis in bone), ii) there could be a genetic disorder (somatic mosaicism) facilitating tumour formation at multiple locations; iii) the production of multiple circulating growth factors in the bone marrow could lead to vascular proliferation resulting in multifocal malignancy. However, Pansuriya and co-workers identified IDH1 and IDH2 mutations in a large proportion of echondromas and spindle cell haemangiomas[38]. These mutations occurred also in the majority of multiple lesions within one patient[38]. More recently, Antonescu et al. demonstrated the presence of the characteristic WWTR1-CAMTA1 fusion transcript product with the presence of an identical breakpoint in WWTR1 and CAMTA1 in all different tumour nodules of two patients with multicentric epithelioid haemangioendothelioma of the liver[39]. These findings support the hypothesis of clonal disease and suggest that the tumour nodules are metastatic implants, rather than synchronous multiple neoplastic clones[39].

Table 2. Schematic overview of the different classification schemes proposed over the years.

Entities	Wenger et al. 2000 All lesions	O'Connell et al. 2001 Epithelioid lesions	WHO 2002 All lesions	Maclean et al. 2006 Epithelioid and spindle cell shaped lesions	WHO 2013 All lesions
Haemangioma	Haemangioma	n.r	Haemangiomas and related lesions	n.r.	Haemangioma
Epithelioid haemangioma	Epithelioid haemangioma	Epithelioid haemangioma		n.r.	Epithelioid haemangioma
Spindle cell haemangioma	n.e.	n.e		Spindle cell haemangioma	n.e.
Haemorrhagic epithelioid and spindle cell haemangioma	n.e.	n.e.		Haemorrhagic epithelioid and spindle cell haemangioma	n.e.
Haemangioendothelioma	n.e.	n.e.		Haemangioendothelioma	n.e.
Haemangiomatosis	Haemangiomatosis	n.e		n.r.	n.e.
Hobnail haemangioendothelioma	n.e.	n.e	n.e	Hobnail haemangioendothelioma	n.e.
Kaposiform haemangioendothelioma	n.e.	n.e.	n.e	Kaposiform haemangioendothelioma	n.e.
Epithelioid haemangioendothelioma	Epithelioid haemangioendothelioma	Epithelioid haemangioendothelioma	Angiosarcoma	Epithelioid haemangioendothelioma	Epithelioid haemangioendothelioma
Haemangioendothelioma, low grade	Haemangioendothelioma	n.r		n.r.	Angiosarcoma
Haemangioendothelial sarcoma, low grade		n.r		n.r.	
Angiosarcoma, low grade		n.r		n.r.	
Haemangioendothelioma, high grade	Angiosarcoma	n.r		n.r.	
Haemangioendothelial sarcoma, high grade		n.r		n.r.	
Angiosarcoma, high grade		n.r		n.r.	
Epithelioid angiosarcoma	n.e.	Epithelioid angiosarcoma	n.e.	Epithelioid angiosarcoma	
Kaposi sarcoma	n.e.	n.e.	n.e.	Kaposi sarcoma	n.e.

n.e. non-exsisting entity in this classification
n.r. not relevant in this classification

VI. Molecular alterations in vascular tumours

So far, only few studies focussed on molecular genetic changes in vascular tumours of bone. The identification of the molecular genetic background could help to better classify these vascular tumours of bone and provide new therapeutic options to improve survival and prognosis. For its soft tissue counterpart, few of the molecular genetic changes are known. Over the past years, researchers have shown a great interest in vascular tumours, mostly of soft tissue, resulting into new insights in molecular changes and a possible role in tumourigenesis. Genetic analysis of angiosarcomas of soft tissue, which has been mostly single case reports or publications of very small tumour groups, showed complex genomic aberrations suggesting angiosarcoma belongs to the groups of sarcomas with a complex genetic profile. In this context the retinoblastoma (Rb) pathway or TP53 pathway is most often involved. Therefore, some authors have suggested p53 gene mutations[40,41] and chromosomal anomalies to be essential in their development. Although nearly 50% of the angiosarcomas showed p53 overexpression by immunohistochemistry, only in 4% a p53 mutation was detected[42]. Moreover, Antonescu and colleagues identified a subset (10%) of angiosarcomas to contain *KDR* (*kinase insert domain receptor* encoding for VEGFR2) mutations which correlated with a strong KDR protein expression and breast localisation[42,43]. Furthermore, high level MYC amplification was shown in radiation-induced and lymphedema-associated angiosarcoma and not in primary angiosarcomas or radiation-induced atypical vascular lesions[42-45], suggesting a possible role in tumourigenesis specifically in secondary angiosarcomas. In 25% of the secondary angiosarcomas FLT4 co-amplification was also present[42, 45]. However, recent publications have shown that MYC amplification can also occur in a small subset of primary angiosarcomas[42,46]. A recent whole-genome sequencing study of both spontaneous (primary) and secondary angiosarcomas demonstrated that not all secondary angiosarcomas harbour a MYC amplification, in this study only 11 of 19 well documented secondary angiosarcomas (58%) showed a MYC amplification[47] (Table 3). Moreover, they described two new mutations in secondary angiosarcomas: 45% of the secondary angiosarcomas harboured an inactivating mutation in the *PTPRB* (*VE-PTP*) gene, a negative regulator of angiogenesis by inhibition of vascular endothelial growth factor receptor 2 (VEGFR2), vascular endothelial (VE)-cadherin and angiopoietin signalling. Moreover, three of these secondary angiosarcomas also demonstrated a mutation in *PLCG1*[47]. The *PLCG1* gene encodes for phospholipase Cγ1 (PLCγ1), a tyrosine kinase signal transducer in the PIK3CA signalling pathway. In contrast, only a minority of the spontaneous (primary) angiosarcomas showed genetic alterations in well-known cancer genes, such as PIK3CA, CDKN2A, NRAS, KRAS, and none of these alterations were recurrent[47]. Thus, a significant subset of angiosarcomas, especially breast (KDR) and secondary (FLT4, PTPRB) angiosarcomas demonstrate mutations in genes involved in the regulation of angiogenesis.

Table 3. Overview of the reported genetic aberrations in vascular tumours of soft tissue.

Tumour type	Reported genetic aberrations	Number (%)	Localisation	References
Epithelioid haemangioma	ZFP36-FOSB fusion	20%	bone, soft tissue, penis, lymph node	Antonescu 2014
Pseudomyogenic haemangioendothelioma	SERPINE1-FOSB fusion	1/1 (100%), 2/12 (17%)	soft tissue	Trombetta 2011; Walther 2014
Epithelioid haemangioendothelioma	t(1;3)(p36.3;q25) with fusion gene CAMTA1-WWTR1			Errani 2011
	fusion gene YAP1-TFE3			Antonescu 2013
Angiosarcoma	KDR mutation	4/39 (10%)	breast	Antonescu 2009, Guo 2011
	TP53 mutation	4%	liver	Anonescu 2012
Secundary angiosarcoma	High-level amplification of MYC	11/19 (58%) and 20/20 (100%)		Behjati 2014, Italiano 2012
	Co-amplification of FTL4	1/21 (5%)		Behjati 2014, Italiano 2012
	PTPRB mutation	10/22 (45%)		Behjati 2014
	PLCG1 mutation	3/22 (7%)		Behjati 2014

Over the years, in two epithelioid haemangioendothelioma of soft tissue a translocation t(1;3)(p36.3;q25) had been described, suggesting a recurrent genetic aberration[29,48]. However, only recently the genes involved in this translocation have been identified: *WWTR1* (also known as *TAZ*) on 3q25 fuses with *CAMTA1* on 1p36, resulting in a fusion gene and eventually activating a novel transcriptional program[39]. Moreover, a *YAP1-TFE3* gene fusion has been identified in epithelioid haemangioendothelioma lacking the *WWTR1-CAMTA1* fusion[49]. The latter subgroup seems to have a more distinct morphology including well-formed vessels as compared to classic epithelioid hemangioendothelioma. Although most of these tumours are located within the soft tissue also one of the reported cases was located within the bone (vertebral body)[42,49].

Pseudomyogenic haemangioendothelioma is a more recently described intermediate malignant vascular tumour affecting children and young adults. It is characterized by loose fascicles or sheets of round or oval shaped cells with vesicular nuclei and prominent nucleoli, surrounded by abundant homogeneous eosinophilic cytoplasm[50]. These lesions also have a tendency to occur multifocally. A recent genetic study has identified a balances translocation t(7;19)(q22;q13) in two patient (one lesion of one patient and three lesions of the same patient) and a unbalanced der(7)t(7;19) translocation in another case, resulting in fusion of SERPINE1 and FOSB genes. This entity has been originally described in soft tissue[51,52]. However currently, there have been some reports of primary bone lesions as well[53,54]. Also in a subset (20%) of epithelioid haemangioma, a benign vascular tumour, a ZFP36-FOSB fusion has been detected[55].

VII. Technological challenges/research pitfalls

Since malignant vascular tumours of bone are extremely rare, the collaboration of multiple pathology laboratories- mostly with a special interest in bone and soft tissue pathology – is needed to collect a substantial number of tumours. Many of those laboratories, including our department, had to search within their archives with the consequence that over the years different decalcification techniques had been used which possibly could have an impact on immunohistochemistry or the performed molecular tests. Since the decalcification method never has been specified within the pathology report, it hampers or complicates additional testing, such as reliable immunohistochemical evaluation.

Decalcification of bone is needed in order to be able to cut and evaluate tissue material containing or coming from bone. Decalcification is a chemical process by which calcium hydroxyapatite crystals and other minerals present in bone (or pathological calcifications) dissolve in a decalcification solution, and therefore bone or bony tissue gets the physical characteristics of dense connective tissue[56]. In general, there are three main groups of decalcifying agents:

a. strong mineral acids (e.g. hydrochloric or nitric acid at concentrations up to 10%)
b. weak organic acids (e.g. formic acid, Kristensen buffer)
c. chelating agent (e.g. ethylenediaminetetracetic acid (EDTA))

Strong acids, such as hydrochlorid or nitric acid at concentrations up to 10%, have the advantage of being the most rapid method of decalcification. However, they cause tissue swelling and cellular damage after the usage of 24 to 48 hours and therefore negatively affect the cellular morphology and immunoreactivity of the tissue.
Weak organic acids, such as formic acid, are the most frequently and widely used decalcification agents. Although these acids decalcify more slowly compared to the strong acids, they are gentler and therefore suitable for most routine specimens enabling additional immunohistochemical staining. Since formic acid can damage tissue, and therefore hamper antigens and enzyme histochemical staining, sodium formate (buffered formic acid by Kristensen) can be added as a buffer to counteract the injurious effects of the acid. Moreover, formic acid affects the DNA and leads to defragmentation of the DNA. Ethylene-diamine-tetracid acid (EDTA), a chelating agent, binds metallic ions, such as calcium, on the surface of the apatite crystal thereby slowly reducing its size. This implicates that it is a very slow process, so not suitable for routine diagnostics. However it is a very gentle technique with little tissue and DNA damage and little effect on tissue stainability. Moreover, this method is highly suitable for techniques such as *Fluorescent In Situ Hybridization* (FISH) and Polymerase Chain Reactions (PCR)[56].
Because of the increasing importance of molecular testing, especially within bone and soft tissue tumours and the knowledge of effects of decalcification, EDTA decalcification should be, whenever possible, the preferred method. Although the pathology reports of the material used in this project do not specify the decalcification method, we can assume that most tissue samples are decalcified by either weak organic acids (formic acid) or strong mineral acids.

VIII. Aim of this study and outline of the thesis

The aim of the research described in this thesis is to investigate whether we could determine certain histomorphological characteristics and molecular genetic features which could be helpful in the classification of primary vascular tumours of bone. We compared our dataset of vascular tumours of bone with a small group of angiosarcomas of soft tissue in order to see whether these are truly different tumours or whether they should be regarded as one entity with a different localization.
In **Chapter 2** a detailed summary of the literature and the history of the classification of the different types of primary vascular tumours of bone are presented.
In **Chapter 3** we collected a multi-institute retrospective series of 42 angiosarcomas of bone and investigated their clinicopathological characteristics in relation to outcome.
In **Chapters 4-6** the underlying molecular and genetic changes in primary vascular tumours of bone are investigated. We analysed the well characterized series that we described in chapter 3 using immunohistochemistry to investigate expression of proteins involved in angiogenesis, the cell cycle, or in tumourigenesis in angiosarcoma of soft tissue (**Chapter 4**). For comparison we included a series of angiosarcomas of soft tissue. Because the use of acids during decalcification degrades the DNA and thereby hampers the analysis of genetic aberrations, as described above,

we next optimized the array-Comparative Genomic Hybridization (array-CGH) technique for use on decalcified formalin fixed paraffin embedded tissue in **Chapter 5**. Subsequently, in **Chapter 6** we applied this technique to detect genomic aberrations in angiosarcomas of bone in comparison to a small group of angiosarcoma of soft tissue.

Finally, in **Chapter 7** we question whether the rare vascular tumour previously designated as "haemangiopericytoma of bone" is a true entity or not. We collected a number of primary haemangiopericytoma of bone and re-analysed these lesions in **Chapter 7** using histology, immunohistochemistry and *Fluorescent In Situ Hybridization* (FISH) analysis.

Finally, results are summarized in **Chapter 8** and it is discussed how the results of our studies, integrating morphology and genetics, have contributed to the 2013 WHO classification of vascular tumours of bone.

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