Tenosynovial giant cell tumours

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DIAGNOSTICS

chapter three

Does *CSF1* over-expression or rearrangement influence biological behaviour in tenosynovial giant cell tumours of the knee?

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Abstract

Introduction

Localized- and diffuse-type tenosynovial giant cell tumours (TGCT) are regarded different clinical and radiological TGCT-types. However, genetically and histopathologically they seem indistinguishable. We aimed to correlate *CSF1*-expression and *CSF1*-rearrangement with the biological behaviour of different TGCT-types with clinical outcome (recurrence).

Methods

Along a continuum of extremes, therapy naïve knee TGCT patients with >3 year follow-up, mean age 43(range 6-71)years and 56% female were selected. Nine localized-(two recurrences), 16 diffuse-type(nine recurrences) and four synovitis as control were included. Rearrangement of the *CSF1*-locus was evaluated with split-apart Fluorescence In Situ Hybridization (FISH) probes. Regions were selected to score after identifying *CSF1*-expressing regions, using mRNA ISH with the help of digital correlative microscopy. *CSF1*-rearrangement was considered positive in samples containing >2 split signals/100 nuclei.

Results

Irrespective of TGCT-subtype, all cases showed *CSF1*-expression and in 76% *CSF1*-rearrangement was detected. Quantification of *CSF1*-expressing cells was not informative, due to the extensive intra tumour heterogeneity. Of the four synovitis cases, two also showed *CSF1*-expression, without *CSF1*-rearrangement. No correlation between *CSF1*-expression or rearrangement with clinical subtype and local recurrence was detected. Both localized- and diffuse-TGCT cases showed a scattered distribution in the tissue of *CSF1*-expressing cells.

Conclusion

In diagnosing TGCT, *CSF1* mRNA-ISH in combination with *CSF1* split-apart FISH; using digital correlative microscopy, is an auxiliary diagnostic tool to identify rarely occurring neoplastic cells. This combined approach allowed us to detect *CSF1*-rearrangement in 76% of the TGCT-cases. Neither *CSF1*-expression nor presence of *CSF1*-rearrangement could be associated with the difference in biological behaviour of TGCT.

Introduction

Tenosynovial giant cell tumour (TGCT), previously known as pigmented villonodular synovitis (PVNS) and giant cell tumour of tendon sheath, is a rare, neoplastic lesion arising from the synovial lining of joints, bursae or tendon sheaths in predominantly young adults. Excluding digits, this mono-articular disease is most commonly diagnosed around the knee or other weight bearing joints[1-3].

Initially, TGCT was believed to be an inflammatory disease[4]. After genomic aberrations were discovered, TGCT was evidently considered neoplastic[5-10]. Chromosomal aberrations include trisomy for chromosomes 5 and 7 and translocations involving the short arm of chromosome 1p11-13, most commonly translocated to chromosome 2q37 region. At the 1p13 breakpoint, Colony Stimulating Factor 1 (*CSF1*) gene is located. The translocation leads to a classical promoter fusion event in which collagen 6A3 (COL6A3) promoter element is fused to *CSF1*. As a result, the fusion leads to deregulated expression of *CSF1*[11]. The excessive *CSF1* secretion attracts inflammatory cells that express the *CSF1* receptor (*CSF1*R) (i.e. monocytes and macrophages). Consequently, in TGCT tissue, only a small percentage of cells (2-16%) are neoplastic, carrying the t(1;2) translocation. This phenomenon is coined as "the landscape effect"[11, 12]. Based on *CSF1* rearrangements (translocation), two groups are described. The first group is defined by both *CSF1* over-expression and *CSF1* translocation, whereas the second group lacks the classical translocation. The latter group likely carries other rearrangements altering *CSF1* regulation leading to high *CSF1* mRNA and *CSF1* protein levels[12].

According to the 2013 WHO classification, TGCT is subdivided in a lobulated well circumscribed lesion (localized-type) and a more locally aggressive lesion, involving a large part or all of the synovial lining (diffuse-type)[1, 2, 13] (*figure 1*). Standard choice of treatment was surgical resection of the lesional tissue, either arthroscopically or with an open resection[14-17]. The localized-type TGCT is known with a favourable course after resection (average recurrence rates <6%), while the diffuse-type TGCT generally causes significant morbidity due to the high risk of local recurrence (>50% depending on surgical procedure and follow-up time)[15, 18, 19]. Therefore, at present diffuse-type TGCT is also treated with *CSF1* inhibitors, such as nilotinib, imatinib, pexidartinib, emactuzumab, cabrilazimab and MSC110[20]. Long term efficacy data have not yet been reported with these newer agents.

Recurrent TGCT is rarely lethal, but a chronic illness with substantial morbidity to the joint leading to functional and quality of life impairment, caused by the course of the disease itself and multiple treatments[21]. Clinically, localized- and diffuse-TGCT are clearly two very different diseases. However, histopathologically they seem indistinguishable with both subtypes containing an admixture of mononuclear cells (histiocyte-like and larger cells) and multinucleated giant cells, lipid-laden foamy macrophages (also known as xanthoma cells), siderophages (macrophages including hemosiderin-depositions), stroma with lymphocytic infiltrate and some degree of collagenisation[1, 2].

It remains unclear why localized- and diffuse-TGCT are microscopically and genetically identical, but clinically distinct. Moreover, predictors for progressive disease or local recurrence are lacking. In this study, we investigate whether *CSF1* over-expression and rearrangement are correlated with tumour characteristics (localized-/diffuse-TGCT) and clinical outcome (recurrence). We hypothesize that diffuse-type TGCT, compared with localized-type TGCT, would have a higher load of neoplastic cells. We expect that a higher tumour load is associated with recurrent disease.

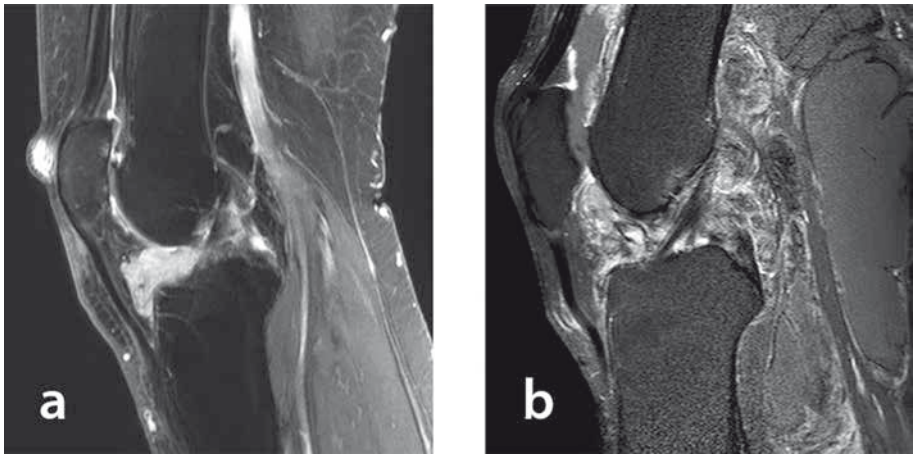


Figure 1 Localized- and diffuse-TGCT sagittal T1-weighted MR image after intravenous contrast injection with fat suppression. Tumour region enhances by contrast injection. **a.** A localized-TGCT involving Hoffa's fat pad in the anterior part of the left knee in a 55-year-old female patient (L4835). **b.** Left knee in a 61-year-old male patient with extensive recurrent diffuse-TGCT located intra- and extra-articular with an additional posterior large Baker's cyst including tumour (L3496).

Methods

Case acquisition and study design

Subtypes of TGCT (localized- or diffuse) were defined based on clinical features and radiological imaging according to 2013 WHO[1, 2]. Along a continuum of extremes, 25 patients with TGCT affecting the knee were carefully selected: patients with small or very large localized or diffuse lesions, with and without recurrent disease. All cases showed all characteristic histological features of TGCT (mononuclear cells, giant cells, macrophages, siderophages, foam cells or lymphocyte-clusters). Included patients were therapy naïve (one diagnostic arthroscopy elsewhere was allowed) and treated with open synovectomy at the Leiden University Medical Centre (LUMC). A clinical follow-up of at least three years was required for inclusion. For comparison, we used tissue specimens of four patients with non-TGCT synovitis. Written informed consent was obtained from all patients. This study was performed in accordance with the Code of Conduct for responsible use in The Netherlands (Dutch Federation of Medical Scientific Societies) and approved by the local medical ethical committee (P13.029).

Inclusion selected cases and tissue specimens

Nine localized- and 16 diffuse-type TGCT patients were included, mean age at surgery of 43 (range 6-71) years, mean follow-up of 57 (range 36-121) months (*table 1*), with a slight female predominance (56%). Two localized- and nine diffuse-type TGCT patients had recurrent disease, after mean 26 (range 14-53) months. The mean age at surgery of the four patients with non-TGCT synovitis was 53 (range 44-65) years, including two (50%) females.

For each patient, multiple formalin-fixed paraffin-embedded (FFPE) tissue blocks and corresponding Haematoxylin and Eosin stained (H&E) 4 µm slides of the primary resected specimen were reviewed by an expert bone and soft tissue pathologist (JVMGB) to confirm TGCT diagnosis and to select representative areas of the tumour with highest proportion of suspected neoplastic cells.

A large tissue heterogeneity was observed between the different blocks. As a control for the landscaped *CSF1* mRNA expression, multiple blocks were selected for three cases (L4046, L3496 and L4954) representing various tissue compositions.

Table 1 Descriptives of study population

	Localized	Localized recurrence	Diffuse	Diffuse recurrence	No TGCT
Total number	7	2	7	9	4
Mean age at surgery (R), y	33 (6-55)	41 (20-62)	54 (33-71)	42 (17-63)	53 (44-65)
Male:female	5:2	0:2	2:5	4:5	2:2
Mean time to recurrence (R), m	na	31 (18;44)	na	24 (14-53)	na
Mean follow up (R), m	61 (39-100)	81 (40;121)	54 (39-97)	51 (36-70)	na

Localized: localized-TGCT; Diffuse: diffuse-TGCT; R: range; y: years; m: months

CSF1 mRNA expression

The RNAscope 2.5 High Definition(HD)-RED assay (Advanced Cell Diagnostics, 322350) was used to detect *CSF1* mRNA expression. This assay visualizes single RNA molecules per cell by a novel method of in situ hybridization (ISH). The double Z probe design allowed simultaneous signal amplification and background suppression[22]. Positive (PPIB (Cyclophilin B)) and negative controls (bacillus subtilis strain SMY) ensured reliable results. mRNA hybridisation were performed according to manufacturer's protocols.

CSF1 rearrangement

To identify the presence of *CSF1* rearrangements at region 1p13, DNA Fluorescence In Situ Hybridisation (FISH) analysis was performed on all tissue specimens using bacterial artificial chromosome (BAC) clones: RP11-354C7 (centromeric to *CSF1*) and RP11-96F24 (telomeric to *CSF1*)) bracketing *CSF1* locus, to identify both translocation and inversion. Probe labelling and hybridisation were done according to previously described protocols[23]. An index case outside of the study population (L4018) was included with a COBRA-FISH molecular karyotyping proven inv(1)(p13;q23) as reference for the detection of the chromosome inversion in tissue section[24]. Detailed description of mRNA ISH and FISH procedures are presented in *supplementary material*.

Scoring and correlative analysis

All slides were scanned in brightfield and/or fluorescence on a Pannoramic P250 or MIDI digital scanner (3DHistech, Budapest, Hungary). Scanned images were visualized using the Pannoramic Viewer (V2.1; 3DHistech). Interpretation was performed manually by a senior FISH expert (KS), blinded towards TGCT-type and clinical outcome. Because *CSF1* expressing regions were expected to contain neoplastic cells, three of these regions were selected. With the use of digital correlative microscopy, regions with *CSF1* mRNA expressing (supposed neoplastic) cells were identified and the same areas were scored after FISH analysis. If the distance between the two signals was larger than the size of a single hybridization signal, cells were recorded *CSF1* split positive. All nuclei within the selected area with a complete set of signals were evaluated. Nuclei with an incomplete set of signals were excluded from counting. Samples containing >2/100 nuclei with a *CSF1* split were considered *CSF1* split positive.

Results

CSF1 mRNA expression

Specimens of all localized- and diffuse-TGCT cases showed a scattered, tissue infiltrating distribution of *CSF1* expressing cells (*figure 2*). Corresponding to the landscape effect, heterogeneous distribution of *CSF1* expressing cells were observed when sections from multiple bock were analysed, meaning that regions completely devoid *CSF1* expressing cells were seen in regions containing large proportion of foam cells or regions with lymphocytic infiltrates. *CSF1* mRNA pattern expression was not observed in multinucleate giant cells, siderophages or foam cells. Consequently, due to the great heterogeneity between different blocks derived from one tumour and within regions in one section, quantification of *CSF1* expressing cells, meaning the expression of the proportion of *CSF1* positive cells, was not informative and was not further analysed (*supplementary material figure 1*). Selecting the block with the highest possible neoplastic cell component, we did not observe a clear difference in distribution of *CSF1* between different TGCT cases. Cells with *CSF1* mRNA expression were distributed diffusely and showed an infiltrating scattered pattern throughout the sections with some clustering at various regions within a tissue element (*figure 2*, *supplementary material figure 2 and 3*).

For the control cases, two of the four cases with synovitis showed expression of *CSF1* (L5619, L5620). However, in these two cases *CSF1* expression was restricted to cells localised in the synovial lining, which was different from the scattered distribution seen in TGCT (*figure 3*). The other two cases with synovitis showed no expression of *CSF1* (L3715, L5622).

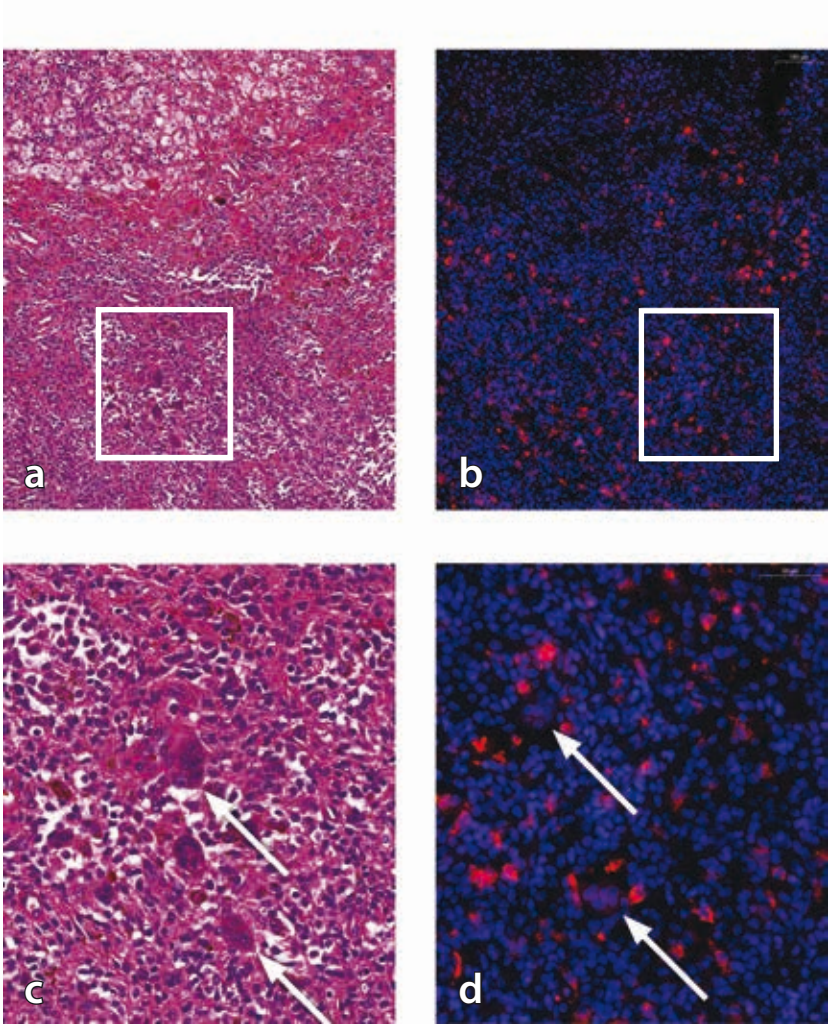


Figure 2 Conventional histology and mRNA ISH from 61-year-old male patient (L3496), with extensive recurrent diffuse-TGCT. This is the same patient as figure 1 right. Left panel H&E stained section (A; C) with matching *CSF1* mRNA ISH (B; D) on the right panel. White box in panel A and B show regions at higher resolution in panel C and D. Heterogeneous cellular composition of TGCT is visible including foam cells, inflammatory cells, synovial-like cells, siderophages and characteristic giant cells (A; C). mRNA ISH shows a scattered distribution of *CSF1* expressing cells with granular cytoplasmic signals (red signal), identifying *CSF1* expressing cell-nuclei (blue signal after DAPI staining). Green arrowheads shows giant cells without *CSF1* expression. Scale bars are in the right top corner 100µm for panel A and B and 50µm for panel C and D.

CSF1 rearrangement

The *CSF1* probe set showed a clear split-apart signal even for detection of chromosome inversion using our molecular karyotyping proven index case with an inv(1)(p13;q23) indicating that cases with no split signal are unlikely to have similar inversion. Due to great heterogeneity, *CSF1* split scoring was done on selected areas based on presence of *CSF1* expressing cells identified by mRNA ISH using correlative digital microscopy. Using this approach, *CSF1*-gene rearrangement was detected in 76% of all TGCT cases: in localized-type 77% and in diffuse-type 75% (*figure 4, supplementary material figure 2*). Further stratification of positive cases, rearrangement of the *CSF1* locus was present in 78% of localized-TGCT without recurrence, 100% of localized-TGCT with recurrent disease, 86% of diffuse-TGCT without recurrence and 67% of diffuse-TGCT including recurrent disease (*table 2, supplementary material table 1 patient and tumour characteristics*). There was no *CSF1* gene rearrangement in all four synovitis control cases.

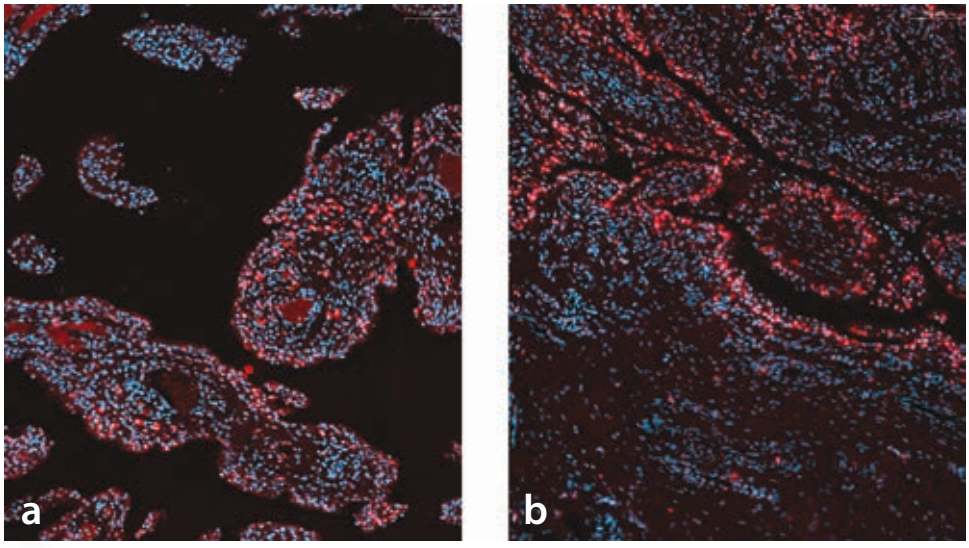


Figure 3 Distribution of synovial lining *CSF1* mRNA ISH positive cells in TGCT and reactive synovitis.
a. 61-year-old male patient (L3496) with diffuse-type TGCT. Cells with red cytoplasmic staining after mRNA ISH, show a deep infiltrating pattern in synovial villi with rare occurrence at the synovial lining parts. This is the same patient as figure 1 right and figure 2. **b.** 45-year-old female patient (L5620) with synovitis, showing *CSF1* expressing cells (red cytoplasmic signal) restricted to cells localised in the synovial lining. Nuclei are displayed in blue after DAPI staining, scale bars are in the right top corner (100µm).

Table 2 Proportion of cases with *CSF1* mRNA expression and *CSF1* gene rearrangement*

	N	*CSF1* **over-expression**	*CSF1* **gene rearrangement**
Localized	7	7 (100%)	5 (78%)
Localized recurrence	2	2 (100%)	2 (100%)
Diffuse	7	7 (100%)	6 (86%)
Diffuse recurrence	9	9 (100%)	6 (67%)
Synovitis	4	2 (50%)	0 (0%)

Localized: Localized-TGCT; Diffuse: Diffuse-TGCT

*Comprehensive patient and tumour characteristics are shown in *supplementary material table 1*.

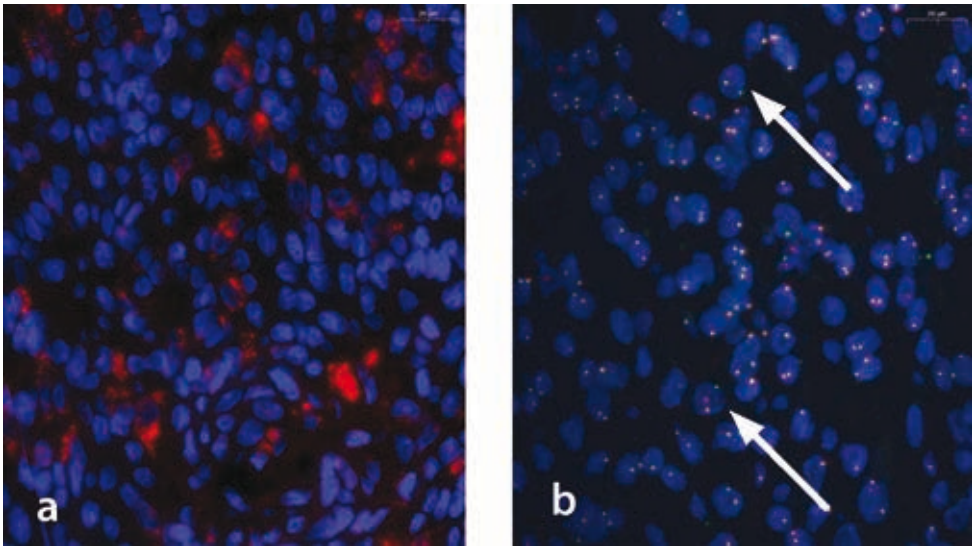
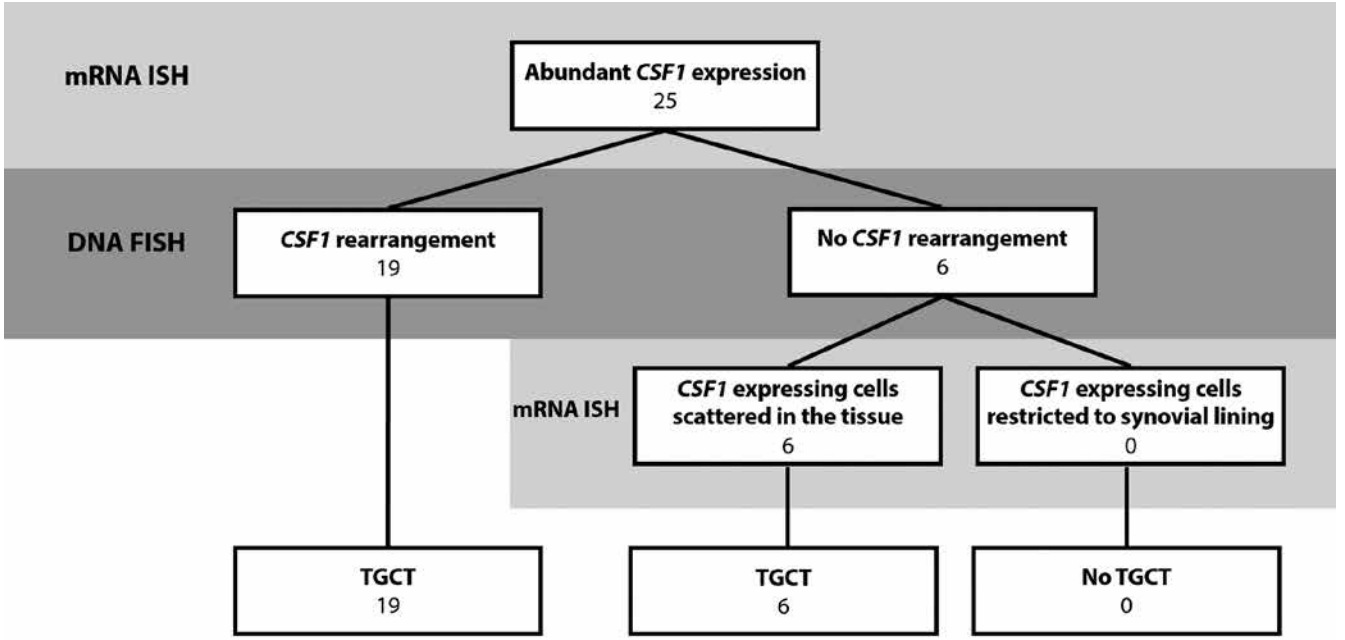


Figure 4 Correlative microscopy used to identify neoplastic cells. **a.** mRNA ISH helps to identify regions with cells overexpressing *CSF1* mRNA (red signal), blue nuclei after DAPI staining. **b.** *CSF1* locus specific split-apart probe set using BAC probes: centromeric (red) and telomeric (green) probes. Yellow signal represent co-localization of the signal meaning no rearrangement. White arrowheads indicate cells with split-apart signal, indicating rearrangement of the *CSF1* gene. Samples are from a 61-year-old male patient (L3496), with extensive recurrent diffuse-TGCT, the same patient as figure 1 A, figure 2, figure 3A. Scale bars are in the right top corner (20µm)

Figure 5 Proposed workflow for molecular pathology work up of TGCT cases

Numbers represent TGCT cases in this study

CSF1, Colony Stimulating Factor1; mRNA ISH, mRNA In Situ Hybridization; DNA FISH, DNA Fluorescence In Situ Hybridisation



Discussion

Localized- and diffuse-type TGCT are histopathologically identical and carry the same chromosomal translocation, leading to uncontrolled over-expression of *CSF1* due to a gene fusion between COL6A3 and *CSF1* genes. Undeniably, localized- and diffuse-type TGCT are clinically different diseases. In a well-defined TGCT population with >3 years follow-up, molecular differences in primary resected tissue between both subtypes and clinical outcome (recurrence) were evaluated. We were unable to find a clear association between *CSF1* over-expression or *CSF1* rearrangement and the biological behaviour in TGCT of the knee.

In this study, 76% *CSF1* rearrangement was detected when lumping all our 25 cases together, compared with 61% of the evaluated cases by Cupp et al.[12]. Further subdivided, our study revealed no difference in *CSF1* rearrangement for localized-TGCT (77%) and diffuse-TGCT (75%). On the contrary, West et al. reported a large difference between these two types; 87% rearrangement in localized- and 35% in diffuse-TGCT[11]. The relatively high percentage of rearrangement in our study, could be attributed to our scoring on preselected areas, based on high *CSF1* expression. In addition, our DNA FISH analysis, using bacterial artificial chromosome (BAC) clones (RP11-354C7 and RP11-96F24) bracketing *CSF1* locus, identifies not only a translocation, but also an inversion for *CSF1* rearrangements. Panagopoulous et al. revealed a *CSF1*-S100A10 fusion gene, with translocation t(1;1)(q21;p11) as the sole karyotypic abnormality[25]. Nilsson et al. found that 30% of the TGCT specimens did not have a rearrangement involving the 1p13 locus, where *CSF1* is located using split-apart interphase FISH approach, similar to ours[8]. Next to the translocation, Panagopoulos et al. reported the replacement of the 3'-UTR of *CSF1*, resulting in over-expression or a longer lifetime of *CSF1* mRNA due to loss of the 3-UTR controlling region[25]. Similar cryptic changes leading to loss of smaller gene region involving the 3'-UTR segment of *CSF1* are beyond the detection level of our FISH probes. Next to this, other, yet not identified alterations leading to deregulated *CSF1* expression cannot be ruled out in cases with *CSF1* mRNA expression without *CSF1* rearrangement of the *CSF1* locus.

Up to date, clinically reliable antibodies working on FFPE tissue sections to detect *CSF1* or *CSF1*R are lacking. Therefore, mRNA ISH was the best regarded option to identify *CSF1* over-expressing cells. Consistent with previous reports, all 25 evaluated cases showed *CSF1* up-regulation[11]. Exact

determination of the proportion of *CSF1* expressing cells was considered not meaningful, since in all tumours considerable intratumoural heterogeneity was observed between selected blocks and with individual tissue sections, reflecting the "landscape effect"[11]. This heterogeneity prevents any conclusion on the true neoplastic cell load in the tumour and a possible correlation to clinical outcome.

Deregulated *CSF1* expression is believed to be the central mechanism of tumourigenesis for TGCT. *CSF1*, also called macrophage colony-stimulating factor, is a cytokine, produced by many different cell types including macrophages, fibroblasts, endothelial cells and osteoblasts (and other cancer types, especially in bone metastasis)[26]. *CSF1* is expressed in neoplastic cells infiltrating throughout the lesion. Secreted *CSF1* recruits non-neoplastic macrophages into the tumour. By binding to its receptor *CSF1*R (type III receptor tyrosine kinase), *CSF1* promotes survival, proliferation and differentiation of cells of the mononuclear phagocyte lineage (e.g. monocytes, macrophages and osteoclasts)[27, 28]. Besides its general biological function, *CSF1* is also involved in inflammatory or reactive synovitis (rheumatoid arthritis, chronic artritis) and cancer (breast, endometrial, ovarian, lung, kidney)[12, 27]. When *CSF1* is expressed in reactive synovitis, its expression is restricted to cells in the synovial lining[12, 29], as was confirmed in our synovitis control cases.

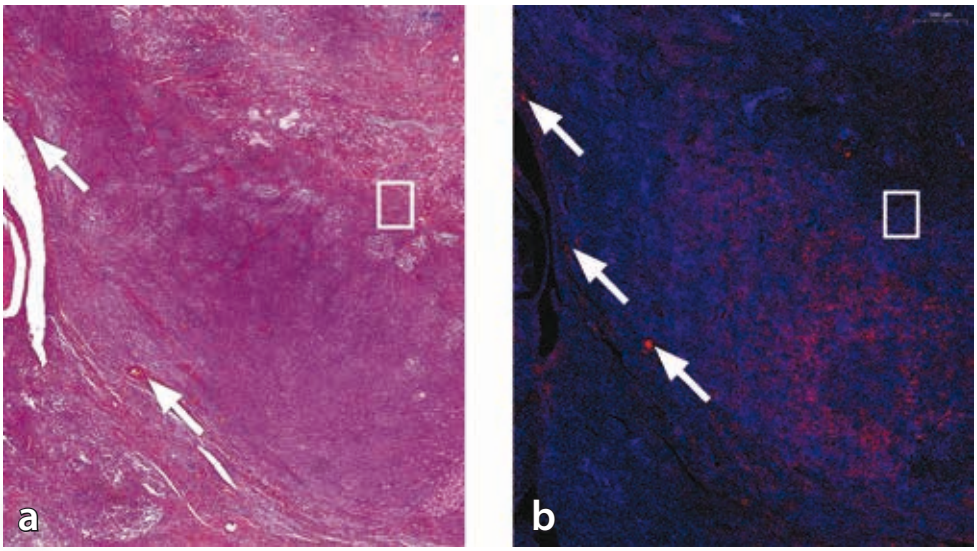
Inhibition of signalling between *CSF1* and *CSF1*R targets the underlying cause of the disease[29, 30]. The involvement of this pathway contributed to the introduction of systemic therapies for extensive diffuse-TGCT[20]. Primarily, imatinib[31] or related drugs as nilotinib[32] showed efficacy in the treatment. Recently, new *CSF1*R blockers were developed and are investigated in clinical trials; Emactuzumab and Cabiralizumab (FPA008) both monoclonal antibodies directed against *CSF1*R[33-35]; Pexidartinib (PLX3397; retains *CSF1*R in inactive state)[29], and MSC110 (an antagonist of the *CSF1* ligand)[35]. Emactuzumab (N=29) showed an overall response rate of 86% (two patients with a complete response) and a rate of disease control of 96%, including a significant functional and symptomatic improvement (median follow up 12 months)[33]. The preliminary results for cabiralizumab (N=22) are consistent, with radiographic response and improvement in pain and function in five out of 11 patients (45%)[34]. In a randomized, placebo-controlled phase 3 study, pexidartinib showed an improved overall response rate by RECIST: 39% in the pexidartinib-group (N=61) and 0% of placebo-group (N=59), after median six months follow-up[36]. However, long term results still need to be evaluated with these newer agents.

Within our well-defined patient cohort, all patients had a minimum follow-up of three years. However, patients without recurrent disease at the time of analysis could still develop this in due course, since it is known that local recurrence might develop years after initial surgery[1, 2, 15, 19, 37]. Verspoor et al. calculated an overall recurrence rate of 72% in 75 patients with diffuse-TGCT of the knee with a mean follow-up from index treatment of 13.9 years. They suggested a trend towards the longer the follow-up, the greater the number of recurrences[19].

In conclusion, DNA FISH analysis, using bacterial artificial chromosome (BAC) clones (RP11-354C7 and RP11-96F24) bracketing *CSF1* locus, can identify both chromosomal rearrangement caused translocation or inversion of the *CSF1* locus. *Figure 5* summarizes the workflow in the current study and the proposed workflow for molecular pathology work up of TGCT cases. The use of *CSF1* mRNA ISH in combination with *CSF1* split-apart FISH is an auxiliary diagnostic tool to confirm the diagnosis of TGCT. This combined approach allowed us to detect *CSF1*-gene rearrangement in 76% of the TGCT cases. At the molecular level, localized- and diffuse-type TGCT are indistinguishable when evaluating *CSF1* expression and the presence of the pathognomonic translocation involving the *CSF1* gene.

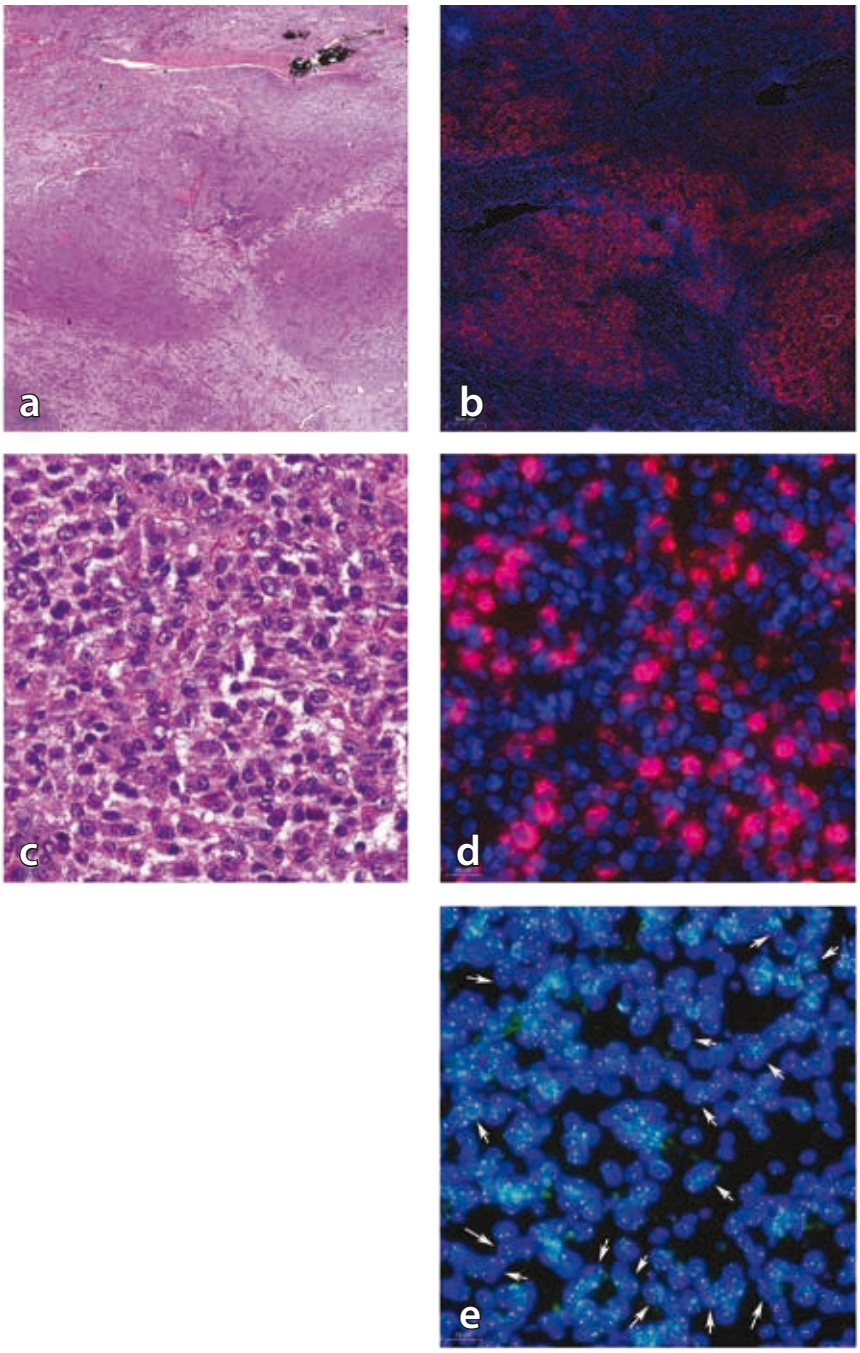
Supplementary data

Supplementary data are available in the online version of this article, doi: 10.1111/his.13744



Supplementary figure 1 Low power magnification overview of TGCT case from a 61-year-old male patient (L3496), the same patient as figure 1 A, figure 2, figure 3A, figure 4. **a.** Hematoxyllin-eosin staining. **b.** *CSF1* mRNA ISH (red) of the same case depicting identical regions, nuclei are stained with DAPI (blue). White arrowheads indicate blood vessels with erythrocytes, giving a strong red fluorescing signal. A heterogeneous distribution of *CSF1* expressing cells with remarkable variation in their distribution in the tissue is clearly visible. White squared inset, indicates regions in high power magnification shown in details in figure 2. Scale bars are in the right top corner (500 µm).

Supplementary figure 2 ***(right page)*** Overview of TGCT localized case without recurrence from a 55-year-old female patient (L4385), presented in figure 1A. **a & b.** Hematoxillin-eosin staining low and high power overview. **b & d.** *CSF1* mRNA ISH (red) of the same case depicting identical regions, nuclei are stained with DAPI (blue). In panel B a white squared inset indicate the region shown in high power magnification in panel D. **e.** Using correlative microscope areas with more neoplastic cells (mRNA ISH positive cells) were identified and scored for *CSF1* locus specific split-apart probe set using BAC probes. Yellow signal represent co-localization of the signal meaning no rearrangement. White arrowheads indicate cells with split-apart signal, indicating rearrangement of the *CSF1* gene. Scale bars are at the bottom left corners and 500 and 20 µm for low and high power images, respectively.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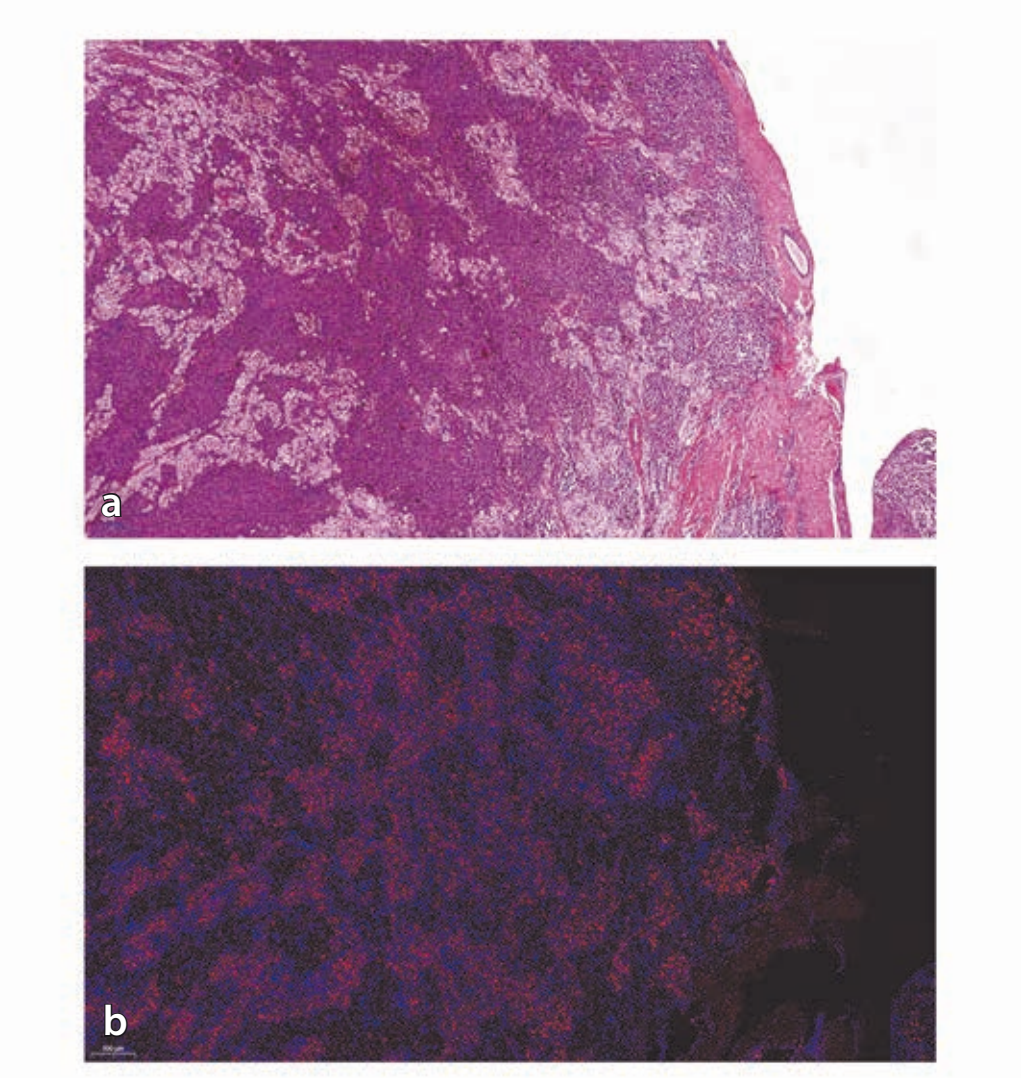

Supplementary figure 3 Correlative microscope image comparing sections after hematoxillin-eosin staining (**a**) and *CSF1* mRNA ISH (**b**) of a diffuse, non-recurrent TGCT case from a 50-year-old male patient (L3697). Diffuse infiltrating *CSF1* expressing cells are present throughout the section. Scale bars are at the left bottom corner (200 µm).

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