Detection of a case of chronic myeloid leukaemia with deletions at the t(9;22) translocation breakpoints by a genome-wide non-invasive prenatal test

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Detection of a case of Chronic Myeloid Leukemia with deletions at the t(9;22) translocation breakpoints by a genome-wide non-invasive prenatal test

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What's already known about this topic?

- Incidental findings occur when undergoing a non-invasive prenatal test (NIPT).
- Incidental findings are not necessarily fetal in origin, but can be maternal as well.
- Aberrant NIPT patterns can be indicative of a maternal cancer, but only very few reports have been published thus far.

What does this study add?

- This study is the first to report that genome-wide NIPT can detect CML caused by the typical t(9;22) translocation when accompanied by deletions at the translocation breakpoints.

No approval of the ethical committee was required for this study.

Abstract

Objective Non-invasive prenatal tests (NIPT) interrogating the complete genome are able to detect not only fetal trisomy 13, 18 or 21, but additionally provide information on other (sub)chromosomal aberrations, that can be fetal or maternal in origin. We demonstrate that in a subset of cases, this information is clinically relevant and should be reported to ensure adequate follow-up.

Method Genome-wide NIPT was carried out and followed by a software analysis pipeline optimised to detect subchromosomal aberrations.

Results The NIPT profile showed deletions on chromosomes 9 and 22: NIPT 9q33.3q34.12(129150001-133750000)x1,22q11.23(23550001-25450000)x1,22q13.1 (37850001-39600000)x1. This result was confirmed by SNP array on maternal genomic DNA, which also demonstrated that the deletions were somatic in nature. FISH and qPCR revealed that the deletions were flanking the translocation breakpoint on the derivative chromosome 9 as the result of a t(9;22)(q34;q11.2) translocation with *BCR-ABL1* fusion typical for Chronic Myeloid Leukemia (CML). Multidisciplinary counseling together with complete blood count taught that the woman was in an early chronic phase CML. The woman was followed up closely and treatment could be postponed until after delivery.

Conclusion Genome-wide NIPT identified a CML in chronic phase caused by the typical t(9;22)(q34;q11.2) translocation and accompanied by deletions flanking the translocation breakpoints.

Introduction

Since its introduction in 2011, the non-invasive prenatal test (NIPT) for the detection of Down syndrome in the fetus has taken off at an unprecedented pace. By 2014, an estimated 1.5 million NIPTs had been performed worldwide (Diana Bianchi, GenomeWeb, June 05 2015), and the number is expected to have increased exponentially in 2015 following the decentralization of the test and the extension to low-risk pregnancies. The advantages of NIPT over other screening tests are unambiguous. Before NIPT, a pregnant woman was dependent on either maternal serum screening or on an invasive test (amniocentesis or chorion villi biopsy). The former is non-invasive like NIPT, but its performance is rather poor[1]. The invasive test has a near 100% specificity and sensitivity, but comes with a low procedure-related risk of a miscarriage[2, 3]. Still, in Belgium, with approximately 130000 pregnancies yearly, procedure-related miscarriage implies the yearly loss of 76 healthy babies[1].

NIPT combines the (almost equally high) specificity and sensitivity of the invasive test with the non-invasiveness of the maternal serum screening and its success therefore comes as no surprise. With NIPT, cell-free fetal DNA (cffDNA) circulating in the mother's blood is being analyzed for aberrations from a reference set of euploid fetuses, represented (in most cases) as the Z-score. However, it should not be forgotten that NIPT is still a screening test, as false-positives and false-negatives do occur. In most cases, this will be due to biological factors like a low fraction of cffDNA, confined placental mosaicism, true fetal mosacism, the presence of a vanishing twin or a maternal aberration (either a copy number variation (CNV) or a mosaic aneuploidy).

Over time, NIPT has expanded from detecting only trisomy 21 to identifying trisomy 18 and 13 as well, but the sensitivity is somewhat lower[4]. Moreover, some companies (eg Sequenom, Natera, Illumina and Ariosa Diagnostics) report sex chromosome aneuploidies and recently, also microdeletions and -duplications.

NIPT can be either targeted or genome-wide. While providing more information, the latter method comes with a higher incidence of incidental findings; moreover, these findings are not necessarily present (only) in the patient (in this case the fetus), but can be present in the mother, since her cell-free DNA (cfDNA) makes up roughly 90% of the total cfDNA isolated from a plasma sample. Indeed, maternal aberrations will be picked up much more easily and at higher resolution than fetal ones. Consequently, genome-wide NIPT raises some important ethical questions on how to counsel pregnant women and on what to report. As in any other genetic test, an optimal balance between findings related to the indication for testing and incidental findings should be kept in mind.

In 2015, the first reports were published on the detection of maternal malignancies following aberrant NIPT profiles[5, 6]. For example, Amant and colleagues[6] detected respectively an ovarian carcinoma, a follicular lymphoma and a Hodgkin lymphoma in 3 women with deviant NIPT profiles out of 4000 NIPTs.

In this study, we describe the first case of a Chronic Myeloid Leukemia (CML) detected by NIPT and caused by a t(9;22)(q34;q11.2) translocation with deletion of both chromosome 9 and 22 derived sequences on the derivative chromosome 9.

Methods

NIPT

cfDNA isolation, library preparation and software analysis were performed essentially as described in Bayindir *et al.*[7] with some minor modifications to the analysis pipeline to automatically detect subchromosomal CNVs larger than 400kb in size. Apart from calculating a Z score per chromosome, Z scores were also calculated per 50kb region. If at least eight consecutive 50kb regions had elevated (Z>1.5) or decreased (Z<-1.5) Z scores, the fold change was calculated, as this provides evidence as to whether the deviation is due to a maternal or a fetal CNV: assuming an average maternal cfDNA fraction of 90%, a maternal CNV is expected to cause a fold change of 0.55 for a heterozygous deletion and 1.45 for a heterozygous duplication. Next-generation sequencing was performed on a NextSeq500 (Illumina, San Diego, CA, USA) using the High Output v2 kit that generates 75bp single reads. Reads were trimmed to 50bp before analysis with the NIPT software pipeline. The reference set consisted of 50 euploid male and 45 euploid female fetuses.

SNP array

Single nucleotide polymorphism (SNP) array analysis to detect deletions and duplications was performed starting from 200ng of genomic DNA extracted from maternal peripheral white blood cells (MAXWELL® 16 Buccal Swab LEV DNA Purification kit, Promega Corporation, Madison, WI, USA) and using a HumanCyto-SNP-12 v2.1 BeadChip on an iScan system following standard protocols as provided by the manufacturer (Illumina). CNV analysis was performed with CNV-WebStore[8].

FISH

FISH analysis was performed on a maternal blood smear according to standard protocols. A *BCR-ABL1* triple-color, dual-fusion probe (Kreatech Diagnostics, Amsterdam, The Netherlands) was used. FISH images were captured using a Leica DMRA microscope with the CW4000 imaging system (Leica Microsystems, Cambridge, UK).

Quantitative real-time PCR

RNA was extracted from maternal peripheral white blood cells using the RNeasy mini kit (Qiagen, Hilden, Germany). A *BCR-ABL1* PCR product was generated with specific primers and probes (*BCR-ABL1* M-bcr FusionQuant kit CE Qiagen) detecting the b3-a2 en b2-a2 fusion transcripts with TaqMan technology on a LightCycler 480 (Roche, Basel, Switzerland) according to the manufacturer's instructions. *ABL1* control gene expression was used for relative quantification. Results are reported as the percentage ratio of fusion gene transcripts to wild-type *ABL1* transcripts (% *BCR-ABL1*/*ABL1*), aligned with the International Scale[9, 10].

Results

NIPT was performed as a first tier screening test at 13 weeks in a 25-year-old secundigravida at low risk for an aneuploidy. The results showed the absence of trisomy 13, 18 and 21. However, Z scores for chromosomes 9 and 22 were -5 and -4.5 respectively. The bin median, which is a measure for aneuploidy[7], was 0 for both chromosomes, suggesting subchromosomal events. This was confirmed by the graphic pattern, clearly showing subchromosomal deletions on chromosomes 9 and 22, with intensities that suggested that they were maternal in origin (Figure 1). Our NIPT pipeline defined the borders of the deletions as follows (GRCh37): chromosome 9: 129150001-133750000; chromosome 22: 23550001-25450000 and 37850001-39600000. Repeat analysis on a second blood sample,

taken 2 weeks later, confirmed the presence of the deletions (resp. Z-scores of -5.5 and -5.9 for chromosomes 9 and 22). One of the deleted regions harbours the *STXBP1* gene. Mutations in *STXBP1* cause early infantile epileptic encephalopathy (# MIM 612164), an early onset disorder characterized by seizures, severe mental retardation with absence of language development and cerebral atrophy. The disorder shows an autosomal dominant mode of inheritance and the predicted mechanism is STXBP1 haploinsufficiency. Therefore deletion of this gene would likely not have gone unnoticed in a 25-year-old individual. This observation led us to believe that the deletions might be somatic in nature. Second, we noted that both *BCR* on chromosome 22q11.2 and *ABL1* on chromosome 9q34 were partially deleted, suggesting the possibility that the deletions might be the result of a somatic t(9;22)(q34;q11.2) translocation with *BCR-ABL1* fusion.

A SNP array on DNA isolated from the maternal white blood cells showed an arr 9q33.3q34.12(129135687-133719750)x1~2, 22q11.23(23633842-25381045)x1~2,22q13.1 (37918672-39553003)x1~2 (Figure 2). These findings were in very close agreement with the NIPT result, and confirmed the somatic nature of the aberrations. Subsequent FISH analysis demonstrated the presence of a t(9;22)(q34;q11.2) translocation with deletions on the derivative chromosome 9 and *BCR-ABL1* fusion in 50% of 100 investigated interphase nuclei (Figure 3). This result was confirmed by quantitative real-time PCR (Table 1).

The findings were communicated to the pregnant woman in a multidisciplinary genetic counseling setting and an urgent hematological work-up was performed. An echographic examination of the abdomen showed hepatomegaly (20 cm) but no splenomegaly (11.9 cm). Initial laboratory results at 17 weeks gestation as well as follow-up values are shown in Table 1. At the time of diagnosis, peripheral blood analysis showed no marked anemia, slightly

elevated platelets and a leukocytosis with mainly neutrophilia. There was no absolute eosinophilia or basophilia but precursor stages were found along with different stages of maturation of neutrophils. There were no blasts detectable in the peripheral blood. Therefore she was staged as chronic phase with a low risk Sokal score (0.51). Given the relatively minor blood anomalies combined with the wish to avoid any risk to the fetus, the patient was monitored closely (every 2 weeks) without intervention. Laboratory results showed an increase in leukocytosis as well as platelet count and myeloid precursors (see Table 1). Since fatigue was her only complaint until delivery, there was no need to initiate therapy. Acetylsalicylic acid was started at 32 weeks to avoid thrombotic complications. At 36 weeks she delivered a healthy baby boy after a Cesarean section. Bone marrow biopsy 2.5 weeks after delivery showed increased cellularity due to granulocytic proliferation with characteristic dwarf megakaryocytes. Treatment with the tyrosine kinase inhibitor (TKI) dasatinib associated with hydroxycarbamide for one month resulted in a fast normalization of the peripheral blood values (Table 1).

Discussion

NIPT is the first genetic test in which the genetic profiles of two individuals (mother and fetus) instead of one are being generated. Consequently, NIPT has the potential to unravel genetic aberrations in the mother despite the test being performed to detect a chromosomal aneuploidy in the fetus. In our department, the informed consent, which needs to be signed by both the referring physician and the pregnant woman, specifies that 'in the rare event of the detection of a maternal abnormality, this will be reported if deemed clinically relevant'.

Maternal cancer is such a clinically relevant incidental finding. In only two papers thus far, comprising four patients in total, cancer has been detected because of unusual aberrations in

the NIPT pattern[5, 6]. This small number of cases most probably reflects the reticence of most (commercial) NIPT providers to disclose results the test was not designed for and/or the lack of consent from the mother to receive this kind of information. Three additional papers describe 13 women diagnosed with cancer who were not informed that their NIPT pattern was aberrant[11-13]. Of note, analyzing deviations on chromosomes 13, 18 and 21 picked up all 13 of those. Our case, with small deletions on chromosomes 9 and 22, would have been missed using the targeted approach, demonstrating the added benefits of interrogating the complete genome.

The incidence of cancer during pregnancy is estimated to be about 0.1%[14]. The most common cancers encountered in women of childbearing age are breast cancer, cervical cancer, Hodgkin's disease, malignant melanoma and leukemia. The incidence of leukemia during gestation is estimated to be around 1 in 75000-100000 pregnancies, and of those, CML accounts for less than 10%. The standard t(9;22)(q34;q11.2) is usually balanced at the cytogenetic level and a retrospective SNP array analysis of five patients diagnosed with CML confirmed that the translocation was balanced even at the molecular level (data not shown). Evidently, the balanced t(9;22)(q34;q11.2) would be missed by the NIPT. However, in approximately 10-15% of CML cases, the translocation is accompanied by deletions on the derivative chromosome 9[15]. These deletions vary in size, but often involve several megabases both on chromosomes 9 and 22, resulting in the loss of the reciprocal ABL-BCR fusion. The prognostic impact of these deletions has been subject of discussion[15]. Initially, they were associated with a poorer outcome but a study from 2010 failed to establish such a correlation [16]. According to recent recommendations by the European LeukemiaNet, deletions on the derivative chromosome 9 do no longer constitute an adverse prognostic factor[9]. The additional 2Mb deletion on 22q13.1, such as seen in our patient, has not been reported in

CML since it is below the resolution of conventional karyotyping. Its significance is therefore unknown.

Due to the teratogenic risk of the drugs used for the treatment of CML (e.g. TKI, hydroxyurea, interferon-alpha,…), this disease is usually treated conservatively during pregnancy. There are some reports of successful treatment of hyperleukocytosis with leukapheresis during pregnancy[17-19]. In the case described here, treatment was postponed until after delivery since the white blood cell count remained relatively low and symptoms were mild.

Prasad[20] pleads against reporting a maternal malignancy picked up by NIPT, because it causes anxiety or even harm (in case an invasive follow-up of the fetus is warranted) and doesn't provide any advantages. For the case reported here, we strongly believe communicating the incidental finding was beneficial to the pregnant woman. No invasive procedure with risk to the fetus was performed, as the translocation was shown to be maternal and somatic in nature. No other invasive tests (eg bone marrow biopsy) were carried out either, as a regular haematological monitoring suffices to keep track of the evolution of the cancer. Although the Sokal score was low at time of diagnosis, CML can progress from a relatively indolent phase into a blast crisis phase; controlling the disease in that phase is much more difficult. The early detection via NIPT allows for proper monitoring and early treatment, commencing right after delivery.

The presence of circulating tumor DNA (ctDNA) in cancer patients holds the promise of ctDNA testing to become the future method of choice for early cancer diagnosis and serial assessment[21]. However, a thorough understanding of, amongst others, the variables influencing the amount of ctDNA and the correlation between tumor and ctDNA abnormalities is required to validate the clinical utility of ctDNA testing.

Conclusion

In conclusion, this study shows discordant NIPT results to be attributable to CML. Early detection of this malignant disease allows the best possible outcome avoiding uncontrolled acceleration or acute transformation.

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Table 1. Haematological profile of the pregnant woman at different gestation times including *BCR-ABL1* values at 17 and 28 weeks of gestation and at delivery. The time points at 19, 24 and 32 weeks are not shown. * after transfusion. n.d. not determined, w: weeks, m: months.

Parameter	Unit	Normal values	Before pregnancy	Pregnancy						After delivery			
			-7m	17w	22w	26w	28w	29w	34w	36w	+ 2w	+2,5w	+4,5w	+6,5w
Hemoglobin	g/dL	12-15	13.4	11.1↓	10.3↓	9.9↓	9.7↓	9.8↓	9.5↓	10.6*↓	8.5↓	9.0↓	9.1↓	9.7↓
Thrombocytes	x10E9/L	140-440	452↑	511↑	546↑	679↑	705↑	693↑	749↑	749↑	1301↑	1588↑	1851↑	146
Leucocytes	x10E9/L	4.3-10	9.7	20.6↑	23.8↑	30.2↑	30.1↑	32.1↑	42.1↑	47.4↑	46.8↑	46.3↑	9.1	4.0
Neutrophils	x10E9/L	2.0-7.0	6.8	15.9↑	18.3↑	21.1↑	20.3↑	23↑	26.4↑	26.3↑	30.0↑	33.6↑	5.7	1.8
Myeloid precursors	x10E9/L	0.0	0.0	0.8	2.0↑	4.4↑	4.4↑	5.0↑	9.6↑	15.5↑	11.5↑	9.3↑	0.0	0.0
Eosinophils	x10E9/L	0.0-0.5	0.09	0.3	0.4	0.6↑	0.5	0.6↑	1.6↑	1.1↑	0.5	0.5	0.3	0.2
Basophils	x10E9/L	0.02-0.1	0.04	0.1	0.3↑	0.5↑	0.3↑	0.4↑	0.8↑	1.3↑	1.2↑	0.9↑	0.1	0.01
BCR-ABL1	/100 *ABL*	0.0	0.0	44.5↑	n.d.	n.d.	44.6↑	n.d.	n.d.	n.d.	n.d.	51.5↑	n.d.	n.d.

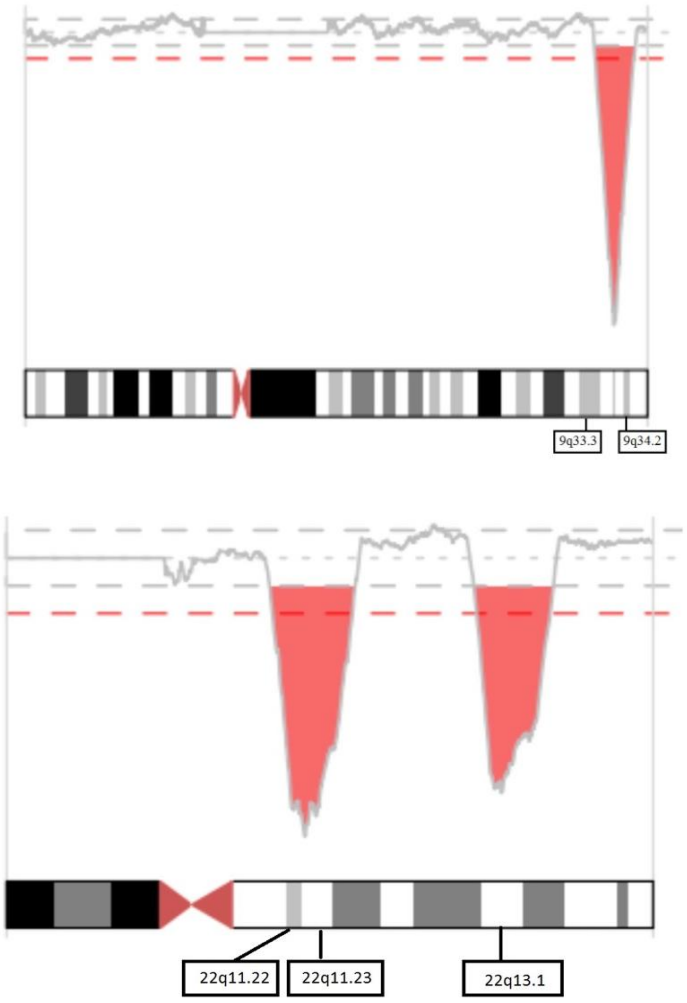

Figure 1. NIPT patterns for chromosome 9 (A) and chromosome 22 (B), clearly showing the deletions as red dips in intensity. Black fine dashed line: Z-score 0; black wide dashed line: Z-score 1.5 or -1.5; red dashed line: Z-score -3.

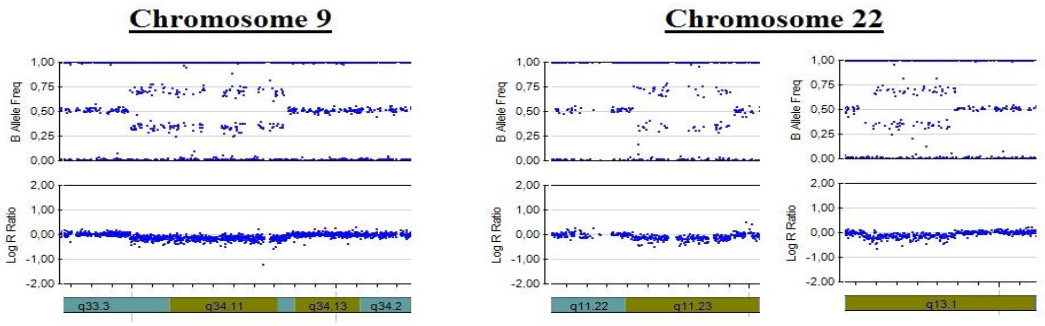

Figure 2. SNP array profiles of the maternal gDNA extracted from white blood cells. Left. Plot of the 9q33.3q34.2 region with split in the B allele frequence and a decreased logR ratio, indicative of a mosaic deletion. Right. Plots of the 22q11.22q11.23 and 22q13.1 regions with 2 splits in the B allele frequency and decreased logR ratios, indicative of 2 mosaic deletions.

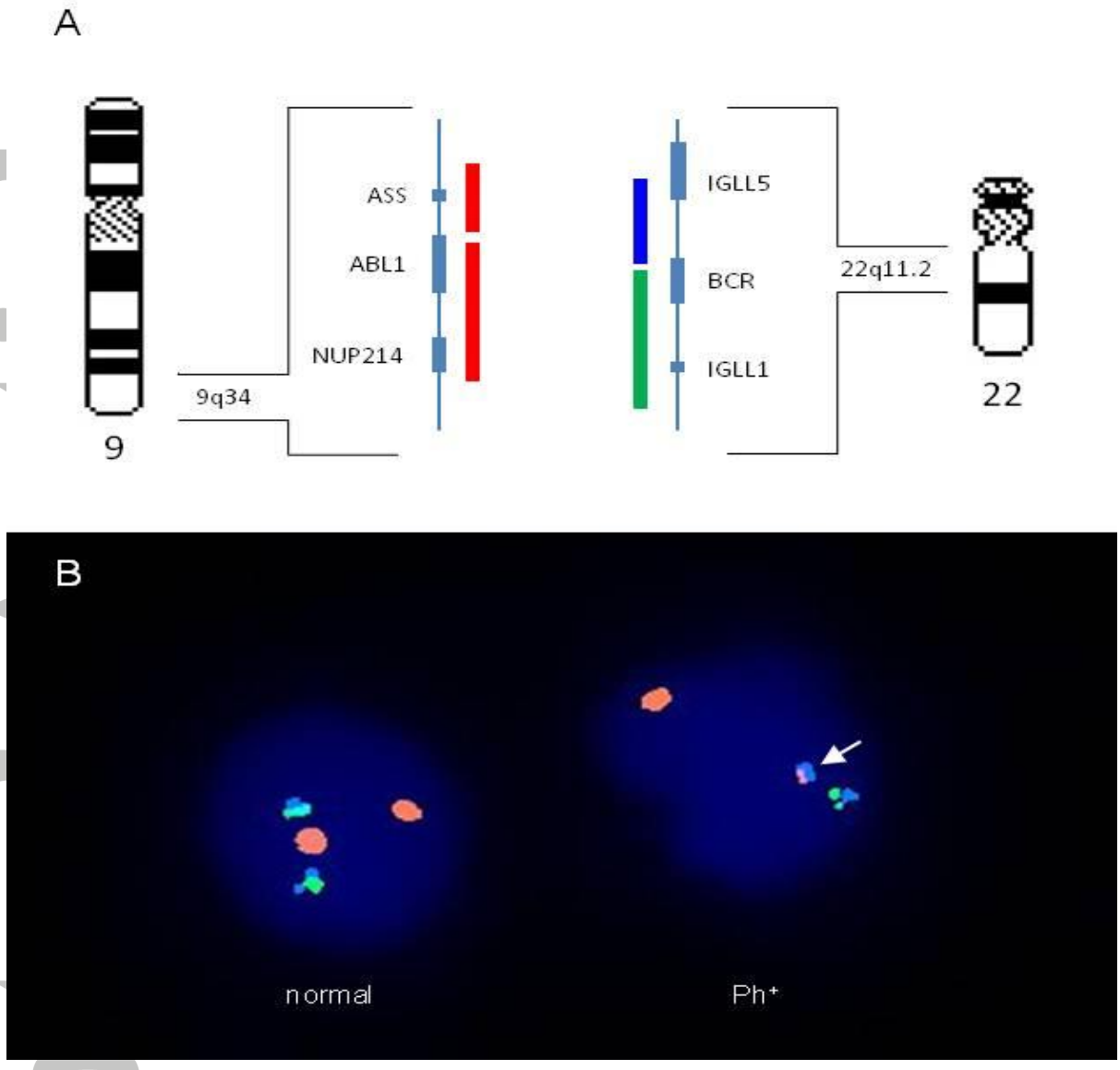

Figure 3 FISH analyses A) *BCR-ABL1* triple color probe design (Kreatech ON BCR-ABL TC fusion) B) Image of a hybridized probe mix on interphase cells. On the left interphase nucleus a normal hybridization pattern is observed (2 orange signals for chromosome 9, 2 blue-green signals for chromosome 22). On the right interphase nucleus an orange/blue fusion signal indicates the derivative chromosome 22 (the so-called Philadelphia chromosome; white arrow), accompanied by a blue/green signal from the normal chromosome 22 and one red signal from the normal chromosome 9. The green/red fusion indicative of a derivative chromosome 9 is missing.