Peripheral blood late mixed chimerism in leucocyte subpopulations following allogeneic stem cell transplantation for childhood malignancies: does it matter?

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In the last decade routine chimerism testing has been established in most transplant centres to verify haematopoietic engraftment but also to guide possible interventions, such as modification of immunosuppression or donor lymphocyte infusions (DLI). Well-established methods of chimerism testing of peripheral blood (PB) or bone marrow (BM) samples include polymerase chain reaction (PCR)-based investigation of variable number tandem repeats (Antin et al, 2001) or short tandem repeats (STR) (Lion, 2003; Schraml et al, 2003; Watzinger et al, 2006; Lion et al, 2012; Clark et al, 2015) for donors of identical gender, and fluorescence in situ hybridization (FISH) of sex-chromosome markers (Kogler et al, 1995; Seong et al, 2000) in case of gender mismatched donor/recipient pairs. Chimerism testing of unsorted leucocytes has a limited sensitivity with a detection limit in the range of one in one hundred cells (10⁻²) when commonly available approaches, such as PCR amplification of microsatellite or STR markers, are applied (Preuner & Lion, 2014). Taking into account that many patients within the first months after HSCT display very low numbers of circulating lymphocytes, it is conceivable that the analysis of fluorescence-activated cell sorting (FACS)-sorted leucocyte subpopulation increases the sensitivity substantially and provides more precise information on engraftment dynamics than analysis of whole blood samples (Lion et al, 2001).

Mixed chimerism (MC), defined as the coexistence of recipient- and donor-derived cell populations, is frequently observed after HSCT following reduced intensity conditioning (RIC) regimens but also after myeloablative conditioning (MAC). Haematopoietic engraftment and chimerism represent a dynamic post-HSCT process, which may be influenced by various factors, such as underlying disease, intensity and...
type of conditioning, graft composition, viral reactivation and post-transplant immunosuppression.

Mixed chimerism has been associated with an increased risk of graft rejection in patients transplanted for severe aplastic and Fanconi anaemia, whereas no impact was reported in patients with thalassemia (Lisini et al, 2008; Lawler et al, 2009). Early post-transplant MC in CD3+ and CD3+/CD56+ cell subsets was linked particularly to graft rejection following RIC and MAC (Matthes-Martin et al, 2003; Breuer et al, 2012).

Early overall complete donor chimerism (CC) and T-cell CC were proposed to be predictive of acute and chronic graft-versus-host disease (α-GvHD and c-GvHD, respectively) (Balon et al, 2005; Park et al, 2011; Rupa-Matyssek et al, 2011; Nikolouis et al, 2013; Elkaim et al, 2014), but data on the chimerism status at the occurrence of GvHD are not the available.

The impact of MC in PB or BM samples on the relapse risk is discussed controversially (Bader et al, 2004a,b; Lamba et al, 2004; Doney et al, 2008; Rettinger et al, 2011; Pochon et al, 2014; Terwey et al, 2014), and an increase of recipient-derived cells in leukaemia-lineage specific subpopulations was reported to predict impending relapse (Gardiner et al, 1998; Zetterquist et al, 2000; Mattsson et al, 2001; Miura et al, 2006). The majority of published observations are based on serial analyses of chimerism between day +14 to day +100 whereas data on late chimerism are scarce (Schaap et al, 2002; Stikvoort et al, 2013). The availability of serial chimerism analyses of FACS-sorted leucocyte subpopulations over a time course of several years post-HSCT prompted us to retrospectively investigate the incidence and the dynamics of late MC, defined as the presence of MC beyond day +50 and up to 12 years after HSCT. We analysed its impact on rejection, c-GvHD and relapse rates in 161 consecutive paediatric haematological patients treated with allogeneic HSCT at our paediatric centre between 2000 and 2013.

**Patients and methods**

**Patient and transplant characteristics**

Between January 2000 and December 2013, 161 consecutive patients with haematological diseases were identified who survived HSCT beyond day +50. Thirteen of these patients underwent subsequent transplantations a median 462 d after their first HSCT (range: 198–989 d) – for disease relapse (n = 12) or secondary leukaemia (n = 1) following the first HSCT – and therefore were censored at the time point of the second transplantation.

The median recipient age was 11-7 years (range: 0-7–26-6 years). The majority of patients was transplanted for acute lymphoblastic or myeloid leukaemia (ALL or AML, respectively) in first, second or subsequent complete remission and received MAC. A 12-Gray total body irradiation (TBI)-based regimen was administered in 86 transplantations, usually in combination with etoposide 60 mg/kg. A predominantly Busulfan-based chemotherapy was used for conditioning according to disease-specific standard European protocols in 75 HSCTs. The primary stem cell source was BM in 124 recipients, while 35 received peripheral stem cells (PBSC). The donors were human leucocyte antigen (HLA)-identical/matched familial donors (MFD) in 61 cases, while 87 recipients were transplanted from matched unrelated donors (MUD), defined by HLA match in at least nine of ten loci. Thirteen patients received grafts from mismatched unrelated donors with two or more HLA mismatches or from haploidentical family donors. High-resolution HLA typing was performed at the allele level for HLA-A, -B, -C, -DR and -DQ in all donor-recipient pairs. Longitudinal lineage-specific chimerism data were available for all patients included. Patient and transplant characteristics are summarized in Table I.

Prophylaxis of GvHD for patients transplanted from unrelated donors consisted of antithymocyte globulin (ATG) Fresenius (Fresenius Biotech, Graefelfing, Germany; 20 mg/kg) or thymoglobulin (Genzyme; Polyclonals S.A.S., Marcy L’Etoile, France; 2.5 mg/kg) given on three consecutive days (days −3 to −1), serum-level-adjusted ciclosporin (CyA) initiated on day −1, and a short-course of methotrexate (MTX) on days +1, +3 and +6. Patients transplanted from MFD received primarily chemophrophylaxis with CyA only, except for eight children treated with CyA plus Mycophenolate mofetil (MMF), and five children in whom a short-course of MTX was added. Eleven MFD recipients received additional serotherapy with thymoglobulin (n = 6), ATG Fresenius (n = 4) or alemtuzumab (n = 1). Three patients transplanted from haploidentical donors received muromonab-CD3 (OKT-3). MMF was administered as chemophrophylaxis in 18 patients as recommended by the respective protocols or according to institutional standards.

**Immunomodulatory measures**

If patients with MC were still under immunosuppression, the respective agents were reduced if no signs of active GvHD were present. The administration of DLI was considered for patients displaying MC despite discontinuation of immunosuppression. However, the administration of DLI was based on the decision of the treating physicians rather than protocol-driven.

**Intervals of chimerism analysis**

Routine chimerism testing on FACS-sorted PB leucocyte subpopulations before day +100 was performed at least once every week. Thereafter, the intervals of analyses in patients with stable MC with donor levels >90% or complete donor chimerism were extended to at least every 2 months during the first year post-transplant, followed by chimerism analyses at least once a year.
Table I. Transplant characteristics (n = 161 HSCT).

| Characteristics               | Number (% or range) |
|-------------------------------|---------------------|
| **Diagnosis**                 |                     |
| ALL                           | 71 (44%)            |
| AML                           | 31 (19%)            |
| CML                           | 14 (9%)             |
| MDS                           | 15 (9%)             |
| JMML                          | 3 (2%)              |
| NHL                           | 12 (8%)             |
| Secondary                     | 15 (9%)             |
| ALL/AML/MDS                   |                     |
| **Recipient age**             | 11.7 (0.7–26.6) years |
| **Gender**                    |                     |
| Female                        | 57 (35%)            |
| Male                          | 104 (65%)           |
| **Conditioning intensity**    |                     |
| Myeloablative                 | 136 (85%)           |
| Reduced Intensity             | 25 (15%)            |
| **Conditioning type**         |                     |
| TBI based                     | 86 (53%)            |
| Chemotherapy based            | 75 (47%)            |
| **Donor**                     |                     |
| Matched familial donor†       | 61 (38%)            |
| Matched unrelated donor        | 87 (54%)            |
| Mismatched donor              | 13 (%)              |
| Mismatched unrelated           | 7                   |
| Mismatched/haploidentical related | 6               |
| **Serotherapy**               |                     |
| ATG Fresenius                 | 53 (33%)            |
| ATG Thymoglobulin             | 51 (31%)            |
| Alemtuzumab                   | 1 (1%)              |
| (Campath)                     |                     |
| OKT-3                         | 3 (2%)              |
| Not administered              | 53 (33%)            |
| **Graft**                     |                     |
| BM                            | 124 (77%)           |
| PBSC                          | 35 (22%)            |
| BM and PBSC                   | 2 (1%)              |
| **Graft composition**         |                     |
| CD34+/kg BW                   | 4.15 × 10^6/kg (0.4–62 × 10^6/kg) |
| CD3+/kg BW                    | 41 × 10^6/kg (0–1920 × 10^6/kg) |

ALL, acute lymphoblastic leukaemia; AML, acute myeloid leukaemia; CML, chronic myeloid leukaemia; MDS, myelodysplastic syndrome; JMML, juvenile myelomonocytic leukaemia; NHL, non-Hodgkin lymphoma; GVHD, graft-versus-host disease; BW, body weight; HSCT, haematopoietic stem cell transplantation; ATG, anti-thymocyte globulin; OKT-3, muromonab-CD3; BM, bone marrow; PBSC, peripheral blood stem cells.

*Including 13 patients >18 years (median 19.6 years; range: 18.1–26.6 years). All but one of these patients were pre-treated in our centre and transplanted for relapsing disease. A 24-year-old patient was referred from an adult centre due to mental retardation for treatment of NHL and received HSCT at the age of 26.6 years after relapse.

†Including one sibling donor with a single HLA mismatch (9/10).

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Patients with increasing recipient MC were monitored more closely until stable MC or CC was documented in at least two consecutive samples.

Late MC was defined as MC after day +50, provided that the number of circulating T-cells at this time point was sufficient for cell sorting and chimerism analysis in all cell-subsets of interest.

**Cell sorting and flow cytometric techniques**

All chimerism analyses were performed on flow-sorted CD45+ PB leucocyte subtypes defined by their unique antigen co-expression. Myeloid cells were CD33+ monocytes and CD15+ granulocytes; T-cells were defined by their positivity for CD3+/CD4+ (helper T-cells) and CD3+/CD8+ (cytotoxic T-cells), NK-cells and B-cells were defined by their CD3−/CD56+ and CD19+ phenotypes, respectively. Cell sorting was performed on a FACSaria instrument (BD Biosciences, San Jose, CA, USA), after eight-colour staining using a lysis- and wash-cell preparation procedure as described previously (Fritsch et al. 1997). The FACSDiVa software (BD Biosciences) was used for data evaluation. All cell types exceeding 1% of nucleated cells were targets for cell sorting. The number of T-cells isolated for subsequent PCR or FISH analysis ranged between 1000 and 15 000. The purity of the flow-sorted leucocyte fractions was usually >98%.

**Chimerism analysis**

If patients were transplanted from gender-mismatched donors (n = 80), the samples for chimerism testing were analysed using Dual-colour FISH. Cells were dropped onto slides, air-dried and fixed with increasing concentrations of ethanol. Dual-colour FISH was performed according to standard procedures with commercially available probes specific for the centromeric and heterochromatic regions of the X and Y chromosomes, respectively. Depending on the number of cells available, up to 500 leucocytes were analysed within each sorted cell fraction.

For donor-recipient pairs with identical gender (n = 81), a quantitative PCR technique was used for chimerism testing. DNA was extracted from nucleated cells using the QIAamp Blood kit (Qiagen, Hilden, Germany). Recipient and donor DNA were tested before transplantation by a panel of highly polymorphic STR markers to select an informative primer set suitable for the monitoring of chimerism during the post-transplant course (Thiede et al. 2001; Schraml et al. 2003). The detection limit was in the range of 1%. For the analysis of patient/donor origin of cells isolated by flow sorting, the amount of DNA serving as template in individual PCR reactions was generally in the range of 1–20 ng. Upon amplification by PCR, the alleles were quantified by two different approaches: (i) gel electrophoresis and video densitometry using the Kodak Digital Science System with the 1D Image Analysis Software (Kodak, Rochester, NY, USA) and, from
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2003 onwards, (ii) capillary electrophoresis and fluorescence-based quantification using the ABI Prism 310 Genetic Analyser (Applied Biosystems, Foster City, CA, USA), as reported (Schrauwl et al, 2003; Lion & Watzinger, 2006; Lion et al, 2012).

Definition of chimerism groups

Complete chimerism. In line with other investigators, complete donor chimerism (CC) was defined by the detection of >95% donor-derived cells by FISH or >95% donor-specific signals by STR-PCR on at least two consecutive analyses for every leucocyte subset (Michallet et al, 2005; Baron & Sandmaier, 2006; Stikvoort et al, 2013).

All chimerism analyses were performed in sorted cell-subsets. The percentage of overall chimerism was calculated on the basis of absolute cell numbers and the respective percentages of donor-cells at time points displaying the lowest level of donor chimerism in the most prevalent cell subset.

Mixed chimerism. Mixed chimerism was defined as the presence of $\geq$5% recipient-derived cells in at least one cell type of the sorted leucocyte subpopulations at two consecutive time points. Patients with MC were subdivided according to the time point of onset:

1. patients with early MC persisting after day +50 and patients with MC arising de novo after day +50;

2. the outcome of MC:

   a) recipients with persisting MC (defined as MC present on days +50, +100, +150 and +200) and
   b) recipients who lost their MC during the course of subsequent analyses;

3. the dynamics of MC:

   a) stable MC,
   b) MC with decreasing autologous cell subsets at least within two consecutive assessments, and
   c) MC with increasing recipient-derived cell subsets.

Definition of outcomes and GvHD

Graft rejection, chronic GvHD and disease relapse were considered the main clinical outcomes of interest for our retrospective analysis. Patients surviving without event were censored at last follow-up, with a median follow-up time of 5–0 years (range: 0–2–13 years). GvHD was scored and classified according to the published NIH consensus criteria (Filipovich et al, 2005).

Statistical analysis

We designed a Cox proportional hazard model with time-dependent covariates to investigate the impact of the appearance of MC (modelled as time-dependent variable) on the occurrence of relapse and c-GvHD with consideration of additional risk factors (Klein et al, 2001a,b).

Cumulative incidences of relapse (CIR) were estimated according to the chimerism status on day +50, +100, +150 and +200 (excluding patients who relapsed before the respective time points or had a shorter follow-up), considering the competing risk of death. Groups were compared by the method of Gray (Gray, 1988; Fine & Gray, 1999). The date of HSCT was taken as starting point for the statistical analysis.

The evaluation of c-GvHD included 157 patients who did not relapse before day +100 and who had a follow-up period of at least 100 d after HSCT. Cumulative incidence of c-GVHD was estimated according to chimerism status at day +100 and considering the competing risks of death, relapse and second HSCT. Groups were compared using the test of Gray (Gray, 1988; Fine & Gray, 1999).

For non time-to-event variables, the Chi-Square test was used to compare groups for categorical variables. The statistical analysis was performed on the Statistical Analysis System (SAS Institute, Cary, NC, USA).

Results

Engraftment and chimerism

All patients achieved a stable neutrophil engraftment ($\geq$0.5 x $10^9$/l between days +11 and +29 (median day +20). A total of 2530 chimerism analyses were performed in 161 patients with a median of 19 chimerism analyses (range: 6–48) in patients with late MC compared to 14 (range: 1–35) in patients with CC. Overall, 42 patients (26%) with late MC were identified. The percentage of patients with MC and CC over time is shown in Fig 1. In 35/42 (83%) patients, MC had already been present on day +50 (continuous MC). Seven patients (17%) developed MC de novo after day +50 with a median onset of 92 d post-transplant (range: 61–438 d).

Eight patients had stable MC and in five patients recipient MC was increasing. In 29 patients, recipient MC decreased over time, and 24 of them converted to CC between days +84 and +322. Conversion to CC occurred spontaneously in 18 patients, after DLI in five patients (administered for increasing MC, predominantly in the T-cell compartment), and after a G-CSF-stimulated PB stem cell boost (administered for increasing MC observed in all leucocyte subsets) in one patient. In four patients, chimerism was undulating with reappearance of MC after conversion to CC. Twenty-one patients displayed on-going MC at last follow-up. The percentage of maximum overall recipient chimerism was calculated based on absolute cell numbers and the respective percentage of recipient-cells, and varied between 88% and 1% (median 6%). In 24/161 patients, overall recipient
chimerism was >5%, and ≥1% in 34/161 patients. As shown in Fig 1, MC would not have been detected in eight patients on day +50, in five on day +100 and in three patients on day +150 by chimerism analysis performed in unsorted PB samples. No rejections were observed amongst these cases, but three patients relapsed (in one case despite having received pre-emptive DLI).

**Patient and transplant characteristics associated with late MC**

As expected, we found higher rates of late MC in patients who received RIC as compared to MAC (36% vs. 24%), although this difference was not statistically significant ($P = 0.188$). Likewise, a non-significant trend towards an increased incidence of late MC was observed after TBI conditioning as compared to chemotherapy-based regimens (30% vs. 20%; $P = 0.137$). Other previously reported risk factors for MC, such as recipient gender, graft source (BM vs. PBSC), number of CD34+ or CD3+ cells transplanted per kg body weight, donor type (MFD vs. MUD vs. MMD) or the application of serotherapy did not impact significantly on the incidence of late MC in our cohort (Table II).

**Chimerism in cell subsets**

Mixed chimerism was most frequently observed in the CD3+ T-cell compartment, which was documented in 38/42 patients (90%). Fourteen patients (33%) showed MC in the NK-cell or B-cell subsets, respectively, and in 16 recipients (38%), the myeloid compartment was affected. Approximately half of the patients (52%) displayed MC confined to the T-cell subset, whereas the remaining patients had patterns of MC involving also other cell populations, as detailed in Table III.

**Donor lymphocyte infusions**

A total of 10 patients received a median of one DLI (range: 1–4) between day +50 and +1095 after HSCT. Indications for DLI were: the detection of persistent MC despite modification of immunosuppression in patients at high risk of disease relapse and/or delayed engraftment of donor-derived CD3+ T-cells ($n = 8$), molecular relapse of chronic myeloid leukaemia (CML) 3 years after HSCT without evidence of MC ($n = 1$, treated successfully with DLI) and ALL relapse 160 d after HSCT in one patient, who received two DLI on days +199 and +211 in attempt to boost graft-versus-leukaemia reaction without success. Of eight patients who received between one and four DLIs for MC between days +50 and +111 (median +70), three displayed persistent MC until last follow-up despite DLI treatment, and 5/8 converted to CC between 34 and 58 d after DLI. One patient relapsed 19 d after DLI. De novo occurrence of c-GvHD was observed in 4/5 patients following conversion to CC between 50 and 168 d after the DLI administration.

**Rejection**

No graft rejections have been observed. Four patients (3%) experienced secondary graft dysfunction with severe neutropenia (neutrophil granulocytes $<0.5 \times 10^9/l$) and hypocellular bone marrow. Chimerism analysis, however, revealed CC in 4/4 patients. All patients were treated successfully with stem cell boosts from the same donors between day +90 and +190.

**Graft-versus-host disease**

The evaluation of c-GvHD was restricted to 157 patients with a follow-up of at least 100 d after HSCT and absence of
relapse within this time period. Forty patients (25%) developed GvHD between days +75 and +349 after transplantation. Four patients were classified as having late onset (n = 2) or recurrent late (n = 2) a-GvHD, and seven patients had persistent late a-GvHD. Grading according to the NIH consensus criteria (Filipovich et al., 2005) included mild GvHD in six patients (4%), moderate in 16 patients (10%) and severe in 18 patients (11%). Twenty-nine of 119 patients (24%) who never displayed any level of MC developed GvHD, compared to 11 of 42 patients (26%) who displayed MC in one or more cell subsets at any time point after day +50. In seven patients with MC, chronic GvHD was diagnosed between six and 155 d after conversion to CC. Five patients developed GvHD with on-going MC, and three of them had persistent MC until the last follow-up. The 1-year incidences of c-GvHD were calculated according to the chimerism status on day +100, and revealed c-GvHD incidences of 20 ± 7% in patients with MC and 18 ± 4% in patients with CC on day +100 (P = 0.734) as shown in Fig 2. A multivariate Cox regression model, taking into account the

Table II. One-year incidence of late mixed chimerism.

| Variable | Total (number) | Late MC§ (number) | Late MC§ (rates) | P-value (chi square) |
|----------|---------------|------------------|-----------------|---------------------|
| Donor type |               |                  |                 |                     |
| MFD      | 61            | 18               | 0.30            | 0.522               |
| MUD      | 87            | 21               | 0.24            |                     |
| MMD      | 13            | 2                | 0.15            |                     |
| Conditioning intensity |         |                  |                 |                     |
| Myeloablative | 136        | 32               | 0.24            | 0.188               |
| Reduced intensity | 25        | 9                | 0.36            |                     |
| Conditioning type |         |                  |                 |                     |
| TBI-based | 86            | 26               | 0.30            | 0.137               |
| Chemotherapy-based | 75         | 15               | 0.20            |                     |
| Graft |               |                  |                 |                     |
| Bone marrow | 124          | 32               | 0.26            | 0.856               |
| Peripheral blood stem cells* | 37          | 9                | 0.24            |                     |
| Serotherapy† |           |                  |                 |                     |
| With serotherapy | 108        | 30               | 0.28            | 0.337               |
| ATG Fresenius | 53           | 14               | 0.26            |                     |
| ATG Thymoglobulin | 51         | 16               | 0.31            |                     |
| Without serotherapy | 53          | 11               | 0.21            |                     |
| CD34+ cells/kg BW |       |                  |                 |                     |
| <3 × 10^9/kg | 49          | 12               | 0.25            | 0.830               |
| ≥3 and <6 × 10^9/kg | 57          | 16               | 0.28            |                     |
| ≥6 × 10^9/kg | 55          | 13               | 0.24            |                     |
| CD3+ cells/kg BW‡ |       |                  |                 |                     |
| <30 × 10^6/kg | 44          | 13               | 0.30            | 0.802               |
| ≥30 and <60 × 10^6/kg | 63         | 15               | 0.24            |                     |
| ≥60 × 10^6/kg | 49          | 13               | 0.27            |                     |

BV, body weight; MC, mixed chimerism; MFD, matched familial donor; MUD, matched unrelated donor; MMD, mismatched donor; TBI, total body irradiation ATG, anti-thymocyte globulin; BW, bodyweight.

*Two out of the 37 patients received peripheral blood stem cells and bone marrow.
†ATG Fresenius (n = 53), ATG Thymoglobulin (n = 51), Alemtuzumab (n = 1), OKT-3 (muromonab-CD3) (n = 3).
‡Data not available in five patients.
§One patient with MC onset at day +438 excluded.

Table III. Involved leucocyte subtypes in 42 patients with mixed chimerism.

| T-cells | NK cells | Mono/granulocytes | B-cells | Number |
|---------|----------|-------------------|---------|--------|
| Yes     | No       | No                | No      | 22     |
| Yes     | Yes      | Yes               | Yes     | 9      |
| Yes     | Yes      | Yes               | No      | 2      |
| Yes     | No       | No                | Yes     | 2      |
| Yes     | No       | Yes               | No      | 2      |
| Yes     | Yes      | No                | Yes     | 1      |
| No      | Yes      | Yes               | Yes     | 1      |
| No      | Yes      | Yes               | No      | 1      |
| No      | No       | Yes               | No      | 1      |
| No      | No       | No                | Yes     | 1      |

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Relapse

One hundred and twenty-three patients (76%) are alive after a median follow-up of 5.0 years (range: 0.2–13 years). Thirty-three patients (20%) relapsed after their first HSCT, 19 of whom died due to refractory or progressive disease. Ten relapsed patients received a successful second HSCT, and were alive at last follow-up, while the remaining patients died from transplant- or disease-related causes after their second HSCT. Of 126 patients who displayed continuous CC between day +50 and the last follow-up, 25 patients (21%) experienced a relapse. The median interval between the last chimerism analysis and relapse was 39 d (range: 4–345 d). Eight of 42 patients (19%) with MC at any time point relapsed. The relapse rates in patients who showed MC at last follow-up (n = 21) were identical to those who had converted to CC (n = 21), either spontaneously or following DLI, with 3/21 relapses in both groups (14%). Relapses in patients with or without MC and the last chimerism analysis prior to relapse are shown in Fig 3. The 3-year CIRs according to the chimerism status on days +50 and +100 after HSCT (excluding patients who relapsed before the respective time points) were 22 ± 4% vs. 22 ± 8% (P = 0.935) for patients with CC compared to those with MC on day +50 and 21 ± 4% vs. 20 ± 7% (P = 0.907) on day +100 (Fig 4A and B). Results obtained for days +150 and +200 were similar (data not shown).

To assess the impact of chimerism dynamics on disease relapse, we compared patients with persistent MC to patients with persistent CC or with CC following transient MC. Again, the results did not differ significantly between the subgroups, with 3-year CIRs of 8 ± 7% in patients with persistent MC and 19 ± 4% in patients with CC or with CC following transient MC (P = 0.960) (Fig 4C). However, due to low patient numbers, no further statistical evaluation was possible for the groups with increasing, stable or decreasing MC.

For exploratory purposes, the 3-year CIRs were estimated for 42 patients with MC on day +50 or later and for 126 patients with CC on day +50 (patients with subsequent switch to MC were censored). For both groups, the 3-year CIRs were identical (21 ± 7% for patients with MC vs. 22 ± 4% for patients with CC). By multivariate analysis, donor type (HSCT from MMD vs. MUD; hazard ratio (HR) 3.1; 95% confidence interval (CI) 1.1–8.5; P = 0.03) and underlying diagnosis (HSCT for AML or MDS as secondary malignancy vs. ALL: HR 4.7; 95% CI 1.5–14.7; P = 0.008; HSCT for AML vs. ALL: HR 3.0; 95% CI 1.2–7.6; P = 0.02) were independent factors associated with relapse in our cohort. A trend towards lower relapse rates was observed in patients who had received serotherapy (HR 0.2; 95% CI 0.03–1.1; P = 0.061). By contrast, neither the chimerism status nor the conditioning regimen correlated significantly with disease relapse (Table V).

Discussion

Chimerism analyses after allogeneic stem cell transplantations have been performed since the 1970s to confirm allogeneic engraftment (Bortin et al, 1971). At present, chimerism testing is performed routinely in most centres in order to examine PB and BM at different time points post-transplant. It was shown that chimerism is not a steady state but rather a dynamic process with increasing or decreasing proportions of donor cells over time (Schaap et al, 2002). Chimerism is not only determined in nucleated cells from whole blood but is increasingly assessed in leucocyte subsets, such as lymphocyte subpopulations, monocytes or granulocytes (Antin et al, 2001; Lion, 2001; Miura et al, 2006; Breuer et al, 2012). This approach provides a higher sensitivity, especially for the T-cell compartment, which frequently has very low cells

Fig 2. One-year cumulative incidence of chronic GvHD according to chimerism status at day +100. CI, cumulative incidence; cGvHD, graft-versus-host disease; CC complete donor chimerism; MC mixed chimerism.
numbers in the first year post-HSCT. In our patient cohort, MC would have been missed in more than 10% of cases (e.g. eight of 35 patients at day +50) if the analyses had only been performed on unsorted material, especially within the first 200 d after HSCT (Fig 1). Consequently, one patient would not have received DLI if chimerism had been analysed in unsorted PB samples. However, DLI was not able to prevent relapse in this case.

As shown earlier, results of chimerism analysis in PB are equivalent to those in BM (Rauwerdink et al, 2012). For this reason, we restricted our retrospective analysis to the chimerism results obtained from flow-sorted PB cell subsets.

Except for a trend for irradiation-containing conditioning regimens, no other risk factors for MC could be identified in our cohort. A possible explanation for the fact that RIC did not correlate with the occurrence of late MC might be because the analysis was restricted to patients with malignant diseases. The patients receiving RIC had been pre-treated with chemotherapy, and were therefore heavily immunosuppressed at the time of HSCT. Our finding, that late MC is observed more frequently following TBI, is in line with earlier reports (Minculescu et al, 2014).

For obvious immunological reasons, it was hypothesized that MC might be associated with (i) an increased risk of

Table IV. Multivariate regression model for chronic GvHD.

| Parameter                      | P-value | Hazard ratio | 95% Hazard ratio confidence limits |
|--------------------------------|---------|--------------|----------------------------------|
| Patient age (vs. >15 years)    | 0.721   | 1.167        | 0.412–3.300                      |
| <10 years                      | 0.771   | 1.483        | 0.545–4.031                      |
| ≥10 and <15 years              | 0.440   | 1.866        | 0.109–6.799                      |
| Donor type (vs. MUD)           | 0.840   | 0.555        | 0.327–7.385                      |
| MFD                            | 0.579   | 0.858        | 0.109–6.799                      |
| MMD                            | 0.884   | 1.483        | 0.412–3.300                      |
| Conditioning (vs. Reduced intensity) | 0.795   | 0.866        | 0.294–2.556                      |
| Myeloablative                  | 0.795   | 0.671        | 0.144–3.134                      |
| Serotherapy (vs. Without serotherapy) | 0.612   | 0.240        | 0.031–1.871                      |
| With serotherapy               | 0.612   | 0.240        | 0.031–1.871                      |
| MC (vs. CC)                    | 0.173   | 0.240        | 0.031–1.871                      |
| MC                             | 0.173   | 0.240        | 0.031–1.871                      |

GvHD, graft-versus-host disease; MFD, matched familial donor; MUD, matched unrelated donor; MMD, mismatched donor; CC, complete donor chimerism; MC, mixed chimerism.

![Fig 3. Relapses in patients with mixed chimerism (MC; UPN 419–719) and complete donor chimerism (CC; UPN 591–751). White bars indicate MC; Grey bars indicate CC; Black crosses indicate the last chimerism analysis before relapse and grey arrows indicate interventions with donor leucocyte infusion. For unique patient number (UPN) 461 and UPN 644, the time points of relapse/last chimerism analysis were at day +1314/+1100 and +1022/+974, respectively.](image-url)
graft rejection (i.e. residual host T-cells rejecting the allo-
genetic stem cells), (ii) a decreased incidence of c-GvHD (based on the assumption that MC represents a state of tol-
erance) and (iii) an increased relapse incidence (due to the lack of graft-versus-leukaemia reaction). The verification of these assumptions might have a substantial impact on immunomodulatory measures, such as modification of immunosuppression or DLI.

Based on the concept that MC can be converted to CC by DLI, several prospective studies reported the inclusion of pre-emptive DLI in cases of persistent MC in order to prevent relapse (Mohamedbhai et al, 2012; Rujkijyanont et al, 2013; Horn et al, 2015). Rujkijyanont et al (2013) reported on 38 paediatric patients with malignant diseases, including seven minimal residual disease (MRD)-positive children who had received DLI for MC, mainly in the context of...
haploidentical transplantation. DLI was followed by conversion to CC in almost 80% of instances, but only 3/7 patients with concomitant MRD-positivity remained in remission without a second HSCT (Rujikiyanont et al, 2013). Of note, eight patients of the abovementioned cohort cleared a concomitant viraemia and converted to CC after administration of DLI, suggesting that the occurrence of MC in these patients might be attributable to the expansion of autologous virus-specific T-cells rather than impending disease relapse (Borchers et al, 2013). Conversion rates from MC to CC in eight patients of the abovementioned cohort cleared a concomitant viraemia and converted to CC after administration of DLI, suggesting that the occurrence of MC in these patients might be attributable to the expansion of autologous virus-specific T-cells rather than impending disease relapse (Borchers et al, 2013). Conversion rates from MC to CC in these patients does not represent a significant risk factor for graft rejection.

Although no data have been published regarding the incidence of c-GvHD with concomitant MC, the association of early CC with an increased risk of c-GvHD was described (Balon et al, 2005; Park et al, 2011; Rupa-Matyszek et al, 2011; Nikolousis et al, 2013; Elkaim et al, 2014). This is in contrast to our findings indicating that neither the history of MC nor the presence of on-going MC seems to protect from c-GvHD. The majority of earlier studies included elderly patients with malignant diseases, in whom RIC regimens are frequently used (Rupa-Matyszek et al, 2011; Nikolousis et al, 2013). It is therefore tempting to speculate that the reduced toxicity, which is associated with early MC in many cases or other hitherto unknown factors, such as chimerism of dendritic cells, have contributed to the decreased risk of c-GvHD reported in these studies, rather than MC in PB.

The question whether or not MC at any time point post-transplant indicates an increased risk of relapse has been discussed controversially during the last years (Bader et al, 2004a; Lamba et al, 2004; Doney et al, 2008; Rettinger et al, 2011; Terwey et al, 2014). Taking into account the retrospective nature of these studies and the fact that the interval between the detection of recipient cells and relapse was short in many cases, one might speculate that the time points of chimerism testing were driven by the suspicion of impending problems by individual physicians and that, in some cases, the detected autologous cells represented leukaemic blasts, which were below the detection limit of microscopy. In the patient cohort analysed in the present study, detection of MC did not correlate with increased relapse rates. This observation pertained to patients with MC at any time point, patients with a history of MC, and patients with persistent

| Parameter                        | P-value | Hazard ratio | 95% Hazard ratio confidence limits |
|----------------------------------|---------|--------------|-----------------------------------|
| Diagnosis (vs. ALL)              | 0.047   | 3.003        | 1.185–7.614                       |
| AML                              | 0.021   | 0.553        | 0.070–4.354                       |
| CML                              | 0.574   | 1.214        | 0.252–5.844                       |
| MDS/IMML                         | 0.809   | 2.951        | 0.786–11.076                      |
| NHL                              | 0.019   | 4.709        | 1.509–14.699                      |
| Secondary ALL/AML/MDS            | 0.008   |              |                                   |
| Donor type (vs. MUD)             | 0.014   |              |                                   |
| MFD                              | 0.092   | 0.208        | 0.034–1.295                       |
| MMD                              | 0.033   | 3.046        | 1.096–8.468                       |
| Conditioning (vs. reduced intensity) | 0.162     | 2.430        | 0.701–8.423                       |
| Myeloablative                    | 0.162   |              |                                   |
| Serotherapy (vs. Without serotherapy) | 0.061     | 0.175        | 0.028–1.084                       |
| With serotherapy                 | 0.061   |              |                                   |
| MC (vs. CC)                      | 0.105   |              |                                   |
| MC                               | 0.105   | 2.442        | 0.830–7.186                       |

AML: acute myeloid leukaemia; ALL, acute lymphoblastic leukaemia; CML, chronic myeloid leukaemia; MDS, myelodysplastic syndrome; IMML, juvenile myelomonocytic leukemia; NHL, non Hodgkin lymphoma; MFD, matched familial donor; MUD, matched unrelated donor; MMD, mismatched donor; CC, complete donor chimerism; MC mixed chimerism.
MC, indicating that chimerism status in children transplanted for malignant diseases, as assessed in the current study, did not have a significant impact on relapse incidences, in line with the findings of recently published studies (Doney et al, 2008; Nikoloussis et al, 2013; Bernal et al, 2014; Pochon et al, 2014). In addition, taking into account the high incidence of c-GvHD in patients pre-emptively treated with DLI in our cohort and in other published studies, the value of chimerism-guided DLI for the prevention of relapse seems to be questionable (Horn et al, 2015).

Limitations of our study include its retrospective design, the limited size of the cohort, the lack of well-defined time points for chimerism analysis and absence of clearly defined criteria for DLI. We are aware of the fact, that our approach did not include the evaluation of BM and thus CD34+ leucocyte subtypes, as the analysis was restricted to PB samples in which very little if any CD34+ cells are detectable. However, as indicated above, a bias in favour of a positive correlation between MC and relapse would be expected based on the study design. Our data suggest that testing late chimerism in paediatric patients transplanted for malignant diseases does not seem to provide a reliable tool to predict relapse, if performed within the cell subsets described in our analysis. The fact that the detection of MRD post-HSCT is cost effective and indicative of impending leukaemia relapse (Bader et al, 2015), prompted us to restrict late chimerism testing in our centre to children with malignant diseases not amenable to molecular MRD detection (e.g. AML or juvenile myelomonocytic leukaemia) and to specific, well defined prospective research tasks.

In conclusion, the clinical value of late chimerism testing in children undergoing HSCT for treatment of malignant diseases seems to be questionable with regard to the extremely low risk of graft rejection in this setting.

Conflict of interest disclosure

The authors declare no competing financial interests.

Authorship contributions

S. M. and H. P. designed the study and supervised the project; H. P. and S.M. wrote the manuscript with contributions from T. L., G. F., W. H. and O. A. H.; H. P., S. M., S. B., A. L., E. D. G., S. K., E. G. and U. P. were in charge of data pooling, data checking and statistical analysis; G. F. performed the cell sorting and flow cytometric analyses; T. L. and H. D. were responsible for chimerism analysis by STR-PCR; O. A. H. and M. K. performed the FISH analyses; S. M., H. P. and E. G. interpreted the data; All authors read and approved the final version of the manuscript.

References

Antin, J.H., Childs, R., Filipovich, A.H., Giralt, S., Mackinnon, S., Spitzer, T. & Weisfogh, D. (2001) Establishment of complete and mixed donor chimerism after allogeneic lymphohematopoietic transplantation: recommendations from a workshop at the 2001 Tandem Meetings of the International Bone Marrow Transplant Registry and the American Society of Blood and Marrow Transplantation. Biology of Blood and Marrow Transplantation, 7, 473–485.

Bader, P., Kreyenberg, H., Hoelle, W., Dueckers, G., Handgretinger, R., Lang, P., Kremens, B., Dilloo, D., Sykora, K.W., Schrappe, M., Niemeyer, C., Von Stackelberg, A., Grubh, B., Herze, G., Greil, J., Niethammer, D., Dietz, K., Beck, J.F. & Klingebel, T. (2004a) Increasing mixed chimerism is an important prognostic factor for unfavorable outcome in children with acute lymphoblastic leukaemia after allogeneic stem-cell transplantation: possible role for pre-emptive immunotherapy! Journal of Clinical Oncology, 22, 1696–1705.

Bader, P., Kreyenberg, H., Hoelle, W., Dueckers, G., Kremens, B., Dilloo, D., Sykora, K.W., Niemeyer, C., Reinhardt, D., Vormoor, J., Grubh, B., Lang, P., Greil, J., Handgretinger, R., Niethammer, D., Klingebel, T. & Beck, J.F. (2004b) Increasing mixed chimerism defines a high-risk group of childhood acute myelogenous leukaemia patients after allogeneic stem cell transplantation where pre-emptive immuno-therapy may be effective. Bone Marrow Transplantation, 33, 815–821.

Bader, P., Kreyenberg, H., von Stackelberg, A., Eckert, C., Salzmann-Manrique, E., Meisel, R., Poetschger, U., Stachel, D., Schrappe, M., Alten, J., Schrauder, A., Schultz, A., Lang, P., Muller, L., Albert, M.H.I., Willasch, A.M., Klingebel, T.E. & Peters, C. (2015) Monitoring of minimal residual disease after allogeneic stem-cell transplantation in relapsed childhood acute lymphoblastic leukemia allows for the identification of impending relapse: results of the ALL-BFM-SCT 2003 trial. Journal of Clinical Oncology, 33, 1275–1284.

Balon, J., Halaburda, K., Bieniaszewska, M., Reichert, M., Bieniaszewski, L., Piekarzka, A., Pawlowski, R. & Hellmann, A. (2005) Early complete donor hematopoietic chimerism in peripheral blood indicates the risk of extensive graft-versus-host disease. Bone Marrow Transplantation, 35, 1083–1088.

Baron, F. & Sandmaier, B.M. (2006) Chimerism and outcomes after allogeneic hematopoietic cell transplantation following nonmyeloablative conditioning. Leukemia, 20, 1690–1700.

Bernal, T., Diez-Campelo, M., Godoy, V., Rojas, S., Colado, E., Alcocoba, M., Gonzalez, M., Vidriales, B., Sanchez-Guijo, F.M., Lopez-Corral, L., Luno, E. & del Canizo, C. (2014) Role of minimal residual disease and chimerism after reduced-intensity and myeloablative allo-transplantation in acute myeloid leukemia and high-risk myelodysplastic syndrome. Leukemia Research, 38, 551–556.

Borchers, S., Weissinger, E.M., Pabst, B., Ganzenmueller, T., Dammann, E., Luther, S., Diedrich, H., Ganser, A. & Stadler, M. (2013) Expansion of recipient-derived antiviral T cells may influence donor chimerism after allogeneic stem cell transplantation. Transplant Infectious Disease, 15, 627–633.

Bortin, M.M., Saltzstein, E.C., Waixbren, B.A., Kay, S.A., Hong, R. & Bach, F.H. (1971) Bone marrow transplantation for aplastic anemia. Establishment of chimerism using multiple HL-A identical donors following pretreatment with cyclophosphamide. Transplantation, 11, 573–575.

Breuer, S., Preuner, S., Fritsch, G., Duxberger, H., Koenig, M., Poetschger, U., Lwowskiha, A., Peters, C., Mann, G., Lion, T. & Matthes-Martin, S. (2012) Early recipient chimerism testing in the T- and NK-cell lineages for risk assessment of graft rejection in pediatric patients undergoing allogeneic stem cell transplantation. Leukemia, 26, 509–519.

Clark, J.R., Scott, S.D., Jack, A.L., Lee, H., Mason, J., Carter, G.I., Pearce, L., Jackson, T., Clouston, H., Sproud, A., Keen, L., Mollov, K., Folari, N.D., Whitby, L., Snowden, J.A., Reilly, J.T. & Barnett, D. (2015) Monitoring of chimerism following allogeneic hematopoietic stem cell transplantation (HSCT): technical recommenda-
Filipovich, A.H., Weisdorf, D., Pavletic, S., Socie, Doney, K., Loken, M., Bryant, E., Smith, A. & Fritsch, G., Printz, D., Stimpfl, M., Dworzak, Filipovich, A.H., Weisdorf, D., Pavletic, S., Socie, Doney, K., Loken, M., Bryant, E., Smith, A. & Fritsch, G., Printz, D., Stimpfl, M., Dworzak, 916 withdrawal of immunosuppression and donor Haematology versus-host disease: I. Diagnosis and staging Vogelsang, G. & Flowers, M.E. (2005) National arbier model for the subdistribution of a compet- 144 transplants. Part 2: regression modeling. Bone Marrow Transplantation, 28, 1001–1011. Klein, J.P., Rizzo, J.D., Zhang, M.J. & Keiding, N. (2001b) Statistical methods for the analysis and presentation of the results of bone marrow transplantations. Part 2: regression modeling. Bone Marrow Transplantation, 28, 909–915. Kogler, G., Wolf, H.H., Heyll, A., Arkestein, G. & Wernet, P. (1995) Detection of mixed chimerism and leukemic relapse after allogeneic bone marrow transplantation in subpopulations of leukocytes by fluorescence in situ hybridization in combination with the simultaneous immunophenotypic analysis of interphase cells. Bone Marrow Transplantation, 15, 41–48. Lamba, R., Abella, E., Kukuruga, D., Klein, J., Savasan, S., Abidi, M.H., Mohamed, A. & Perse, E. (2004) Mixed hematopoietic chimerism at day 90 following allogeneic myeloablative stem cell transplantation is a predictor of relapse and survival. Leukemia, 18, 1681–1686. Lawler, M., McCann, S.R., Marsh, J.C., Ljungman, P., Hows, J., Vandenberghe, E., O’Riordan, J., Locasciulli, A., Socie, G., Kelly, A., Schrezenmeier, H., Marin, P., Tichelli, A., Passweg, J.R., Dickenson, A., Ryan, J. & Bacigalupo, A. (2009) Serial chimerism analyses indicate that mixed haemopoietic chimerism influences the probability of graft rejection and disease recurrence following allogeneic stem cell transplantation (SCT) for severe aplastic anaemia (SAA): indication for routine assessment of chimerism post SCT for SAA. British Journal of Haematology, 144, 933–945. Lion, T. (2001) Chimerism testing after allogeneic stem cell transplantation: importance of timing and optimal technique for testing in different clinical-biological situations. Leukemia, 15, 292. Lion, T. (2003) Summary: reports on quantitative analysis of chimerism after allogeneic stem cell transplantation by PCR amplification of microsatellite markers and capillary electrophoresis with fluorescence detection. Leukemia, 17, 252–254. Lion, T. & Watzingger, F. (2006) Chimerism analysis following nonmyeloablative stem cell transplantation. Methods in Molecular Medicine, 125, 275–295. Lion, T., Daxberger, H., Dubovsky, J., Filippic, F., Fritsch, G., Printz, D., Peters, C., Matthes-Ma- rin, S., Lawitschka, A. & Gadner, H. (2001) Analysis of chimerism within specific leukocyte subsets for detection of residual or recurrent leukemia in pediatric patients after allogeneic stem cell stem cell transplantation. Leukemia, 15, 307–310. Lion, T., Watzinger, F., Preuner, S., Kreyenberg, H., Tilanus, M., de Weger, R., van Loon, J., de Vries, L., Cave, H., Acquaviva, C., Lawler, M., Brampe, M., Serra, A., Saglio, B., Colnaghi, F., Biondi, A., van Dongen, J.J., van der Burg, M., Gonzalez, M., Alcocoba, M., Barbany, G., Hermanos, M., Roosnek, E., Steward, C., Harvey, J., Frommet, F. & Bader, P. (2012) The Euro-Chimerism concept for a standardized approach to chimerism analysis after allogeneic stem cell transplantation. Leukemia, 26, 1821–1828. Lionetti, D., Zecca, M., Giorgigliani, G., Montagna, D., Cristantelli, R., Labirio, M., Grignani, P., Peveride, C., Di Cesare-Merlone, A., Amendola, G., Bergami, E., Mastronuzzi, A., Maccario, R. & Locatelli, F. (2008) Donor/recipient mixed chimerism does not predict graft failure in children with beta-thalassemia given an allogeneic cord blood transplant from an HLA-identical sibling. Haematologica, 93, 1859–1867. Matthies-Martin, S., Lion, T., Haas, O.A., Frommet, F., Daxberger, H., Konig, M., Printz, D., Scharner, D., Eichstitt, C., Peters, G., Lawitschka, A., Gadner, H. & Fritsch, G. (2003) Lineage-spe cific chimaerism after stem cell transplantation in children following reduced intensity conditioning: potential predictive value of NK cell chimaerism for late graft rejection. Leukemia, 17, 1934–1942. Mattsson, J., Uzumel, M., Tammik, L., Aschan, J. & Ringdén, O. (2001) Leukemia lineage-specific chimerism analysis is a sensitive predictor of relapse in patients with acute myeloid leukemia and myelodysplastic syndrome after allogeneic stem cell transplantation. Leukemia, 15, 1976–1985. Michallet, A.S., Forst, S., Le, Q.H., Dubois, V., Praz, A., Nicolini, F., Thomas, X., Rafii, H., Gebuhrer, L. & Michallet, M. (2005) Impact of chimaerism analysis and kinetics on allogeneic haematopoietic stem cell transplantation outcome after conventional and reduced-intensity conditioning regimens. British Journal of Haematology, 128, 676–689. Mincuclescu, L., Madsen, H.O. & Sengelov, H. (2014) T-cell chimerism is valuable in predicting early mortality in steroid-resistant acute graft-versus-host disease after myeloablative allogeneic stem cell transplantation. Acta Haematologica, 132, 187–192. Minza, Y., Tanaka, J., Toubai, T., Tsutsumi, Y., Kato, N., Hirate, D., Kaji, M., Sugita, J., Shige- matsu, A., Iwao, N., Ota, S., Masuzi, N., Fukuhara, T., Kazai, M., Asaka, M. & Imamura, M. (2006) Analysis of donor-type chimerism in lineage-specific cell populations after allogeneic myeloablative and non-myeloablative stem cell transplantation. Bone Marrow Transplantation, 37, 837–843. Mohamedbhai, S.G., Edwards, N., Morris, E.C., Mackinnon, S., Thomson, K.J. & Peggs, K.S. (2012) Predominant or complete recipient T-cell chimerism following alemtuzumab-based allo genic transplantation is reversed by donor lymphocytes and not associated with graft fail ure. British Journal of Haematology, 156, 516–522.
Nikolousis, E., Robinson, S., Nagra, S., Brookes, C., Kinsella, F., Tauro, S., Jeffries, S., Griffiths, M., Mahendra, P., Cook, M., Paneesha, S., Lovell, R., Kishore, B., Chaganti, S., Malladi, R., Raghavan, M., Mous, P., Milligan, D. & Craddock, C. (2013) Post-transplant T cell chimerism predicts graft versus host disease but not disease relapse in patients undergoing an alemtuzumab based reduced intensity conditioned allogeneic transplant. Leukemia Research, 37, 561–565.

Piek, M., Koh, K.N., Seo, I.J. & Im, H.J. (2011) Clinical implications of chimerism after allogeneic hematopoietic stem cell transplantation in children with non-malignant diseases. Korean Journal of Hematology, 46, 258–264.

Park, C., Oger, E., Michel, G., Dalle, J., Salmon, A., Nelken, B., Bertrand, Y., Cave, H., Cayuela, J., Gravel, N., Macintyre, E., Margueritte, G., Mechinas, F., Robrlich, P., Paillard, C., Demeneoq, F., Schneider, P., Plantaz, D., Poiree, M., Eliaou, J., Semana, G., Druanat, S., Jonveaux, P., Bordigoni, P. & Gandemer, V. (2014) Follow-up of post-transplant minimal residual disease and chimerism in childhood lymphoblastic leukaemia: 90 d to react. British Journal of Haematology, 169, 249–261.

Preuner, S. & Lion, T. (2014) Post-transplant monitoring of chimerism by lineage-specific analysis. Methods in Molecular Biology, 1109, 271–291.

Rauwerdink, C.A., Tsongalis, G.J., Tosteson, T.D., Hill, J.M. & Meehan, K.R. (2012) The practical application of chimerism analyses in allogeneic stem cell transplant recipients: blood chimerism is equivalent to marrow chimerism. Experimental and Molecular Pathology, 93, 339–344.

Rettinger, E., Willachs, A.M., Kreyenberg, H., Bor-Rkhart, A., Holter, W., Kremens, B., Strahm, B., Woessmann, W., Mauz-Koerholz, C., Gruhn, B., Burdach, S., Albert, M.H., Schlegel, P.G., Klingebiel, T. & Bader, P. (2011) Preemptive immunotherapy in childhood acute myeloid leukaemia for patients showing evidence of mixed chimerism after allogeneic stem cell transplantation. Blood, 118, 5681–5688.

Rujkiyanont, P., Morris, C., Kang, G., Gan, K., Hartford, C., Triplett, B., Dallas, M., Sriniwasan, A., Shook, D., Pillai, A., Pui, C.H. & Leung, W. (2013) Risk-adapted donor lymphocyte infusion based on chimerism and donor source in pediatric leukemia. Blood Cancer Journal, 3, e137.

Rupa-Matyssek, J., Lewandowski, K., Nowak, W., Sawinski, K., Gil, L. & Komarnicki, M. (2011) Correlation between the kinetics of CD3+ chimerism and the incidence of graft-versus-host disease in patients undergoing allogeneic hematopoietic stem cell transplantation. Transplant Proceedings, 43, 1915–1923.

Schaap, N., Schattenberg, A., Mensink, E., Preijers, F., Hillegers, M., Knops, R., Pennings, A., Boezeman, J., Geurts van Kessel, A., de Pauw, B. & de Witte, T. (2002) Long-term follow-up of persisting mixed chimerism after partially T cell-depleted allogeneic stem cell transplantation. Leukemia, 16, 13–21.

Schrafl, E., Daxberger, H., Watzinger, F. & Lion, T. (2003) Quantitative analysis of chimerism after allogeneic stem cell transplantation by PCR amplification of microsatellite markers and capillary electrophoresis with fluorescence detection: the Vienna experience. Leukemia, 17, 224–227.

Seong, C.M., Giralt, S., Kantarjian, H., Xu, J., Swankowski, J., Hayes, K., Glassman, A.B., Khouri, I., Korbling, M., Thall, P., Siciliano, M.J. & Champlin, R.E. (2000) Early detection of relapse by hypermetaphase fluorescence in situ hybridization after allogeneic bone marrow transplantation for chronic myeloid leukemia. Journal of Clinical Oncology, 18, 1831–1836.

Stikvoort, A., Gertow, J., Sundin, M., Remberger, M., Mattsson, J. & Uhlin, M. (2013) Chimerism patterns of long-term stable mixed chimeras posthematopoietic stem cell transplantation in patients with nonmalignant diseases: follow-up of long-term stable mixed chimerism patients. Biology of Blood and Marrow Transplantation, 19, 838–844.

Terwey, T.H., Hemmati, P.G., Nagy, M., Pfeifer, H., Golkbujet, N., Bruggemann, M., Le Duc, T.M., le Coutre, P., Dorken, B. & Arnold, R. (2014) Comparison of chimerism and minimal residual disease monitoring for relapse prediction after allogeneic stem cell transplantation for adult acute lymphoblastic leukemia. Biology of Blood and Marrow Transplantation, 20, 1522–1529.

Thiede, C., Bornhauser, M., Oelschlägel, U., Brendel, C., Leo, R., Daxberger, H., Mohr, B., Florek, M., Kroschinsky, F., Geissler, G., Naumann, R., Ritter, M., Prange-Krex, G., Lion, T., Neubauer, A. & Ehninger, G. (2001) Sequential monitoring of chimerism and detection of minimal residual disease after allogeneic blood stem cell transplantation (BSCT) using multiplex PCR amplification of short tandem repeat-markers. Leukemia, 15, 293–302.

Watzinger, F., Lion, T., Steward, C. & Eurochimerism, C. (2006) The RSD code: proposal for a nomenclature of allelic configurations in STR-PCR-based chimerism testing after allogeneic stem cell transplantation. Leukemia, 20, 1448–1452.

Zetterquist, H., Mattsson, J., Uzunel, M., Nasman-Bjork, I., Svensberg, P., Tammik, L., Bayat, G., Winärski, J. & Ringdén, O. (2000) Mixed chimerism in the B cell lineage is a rapid and sensitive indicator of minimal residual disease in bone marrow transplant recipients with pre-B cell acute lymphoblastic leukaemia. Bone Marrow Transplantation, 25, 843–851.