

## CHAPTER 4

### FINDINGS

#### 4.1 Characteristics of Patients with ALL with *BCR-ABL1* in Hospital Ampang

A total of 96 adult patients with a female preponderance were identified (56.3%). The median age of transplantation was 37.5 years (range 14-69 years). Eight patients (8.3%) were in the adolescent age group of 12 to 19 years, while 45 patients (46.9%) were in the young adults age group of 20 to 39 years. The remaining 44.8% (n=43) were patients aged 40 years and older. The majority of patients were of the Malay ethnic group (50%, n=48) followed by Chinese (38.5%, n=37) and Indian (14%, n=19). 53.1% of the patients (n = 96) had a high white cell count ( $>30 \times 10^3 / \mu\text{L}$ ). The median white blood cell count and platelet was  $36 \times 10^3 / \mu\text{L}$  (Table 3).

#### 4.2 Treatment Modalities and *BCR-ABL1* Monitoring

A total of 49 adult patients received the GMALL / BEM protocol (51%) while the remaining 47 adult patients (49%) received the HyperCVAD protocol. A total of 38 patients (39.6%) who had completed chemotherapy and were transplant eligible underwent stem cell transplantation. The median age of transplant was 37.5 years old (range 14-69 years old). Blinatumomab was used in 2 patients and CART was used in 8 patients as a reversal therapy prior to allogeneic stem cell transplantation.

Molecular response after induction, TP1 showed *BCR-ABL1*  $\geq 0.01\%$  in 38 patients (67.9%) and *BCR-ABL1*  $< 0.1\%$  in 18 patients (32.1%). Molecular response at post-consolidation week 16, TP2 showed *BCR-ABL1*  $\geq 0.01\%$  in 37 patients (56.9%), *BCR-ABL1*  $< 0.1\%$  in 28 patients (28%). Molecular response at the end of chemotherapy or

pre transplant, TP3 showed *BCR-ABL1*  $\geq 0.01\%$  in 21 patients (42.9%), *BCR-ABL1*  $< 0.1\%$  in 28 patients (57.1%) (Table 4)

Table 3: Baseline patient characteristics

| Baseline characteristics (n=96)  | N (%)        |
|----------------------------------|--------------|
| <b>Age, years</b>                |              |
| Median (range)                   | 37.5 (14-69) |
| Mean                             | 37.85        |
| 12-19 (Adolescent)               | 8 (8.3)      |
| 20- 39 (Young adult)             | 45 (46.9)    |
| 40- 59 (Adults)                  | 38 (39.6)    |
| $\geq 60$ (Elderly)              | 5 (5.2)      |
| <b>Ethnicity</b>                 |              |
| Malay                            | 48 (50.0)    |
| Chinese                          | 37 (38.5)    |
| Indian                           | 9 (9.4)      |
| Others                           | 2 (2.1)      |
| <b>Gender</b>                    |              |
| Male                             | 42 (43.8)    |
| Female                           | 54 (56.3)    |
| <b>WCC</b>                       |              |
| Median (range)                   | 36 (2-500)   |
| Mean                             | 87           |
| $> 30 \times 10^3 / \mu\text{L}$ | 51 (53.1)    |
| $< 30 \times 10^3 / \mu\text{L}$ | 45 (46.9)    |

### 4.3 Outcomes of *BCR-ABL1* Positive B-ALL Patients

A total of 80 patients (83.3%) achieved remission after induction, with 4 deaths (4.3%) occurring during induction chemotherapy. Overall remission for this population was 38 (41.3%). With a median follow-up of 13 months, the median overall survival of 16 months was not reached. As shown in Table 4, overall survival at 2-year, 3-year, and 5-year (OS) was 34.3%, 29.5% and 25.2%, respectively. With a median follow-up of 9 months, the median event-free survival of 11 months was not reached. As shown in Table 4, overall survival at 2-year, 3-year and 5-year event-free survival (DFS) was 31.4%, 28.4% and 24.3% respectively. At the time of analysis, 33 patients were alive (34.4%), while 63 patients had died (65.6%) with the main cause attributed to disease progression (66.1%) as shown in Table 4.

Table 4: Treatment modalities and molecular *BCR-ABL1* monitoring

| Treatment modalities           | N (%)     |
|--------------------------------|-----------|
| <b>Chemotherapy</b>            |           |
| GMALL/BFM protocol             | 49 (51)   |
| HyperCVAD                      | 47 (49)   |
| Transplanted post-chemotherapy | 38 (39.6) |
| <b>*Novel Agent</b>            |           |
| Blinatumumab                   | 2         |
| CAR-T                          | 8         |
| <b>Treatment response</b>      |           |
| Remission post induction       | 80 (83.3) |
| Overall Remission              | 38 (41.3) |
| Relapse                        | 34 (37.0) |
| Refractory                     | 10 (10.9) |

|                                             |            |
|---------------------------------------------|------------|
| Induction death                             | 4 (4.3)    |
| <b>Molecular BCR ABL achieving MMR</b>      |            |
| <b>Post induction MRD</b>                   |            |
| ≥0.01%                                      | 38 (67.9)  |
| <0.01%                                      | 18 (32.1)  |
| <b>Post consolidation week 16</b>           |            |
| ≥0.01%                                      | 37 (56.9)  |
| <0.01%                                      | 28 (43.1)  |
| <b>Post end of treatment/pre-transplant</b> |            |
| ≥0.01%                                      | 21 (42.9)  |
| <0.01%                                      | 28 (57.1)  |
| <b>Overall survival</b>                     |            |
| Median follow up, month (range)             | 13 months  |
| Median overall survival                     | 16 months  |
| 2 years                                     | 34.3%      |
| 3 years                                     | 29.5%      |
| 5 years                                     | 25.2%      |
| <b>Disease Free Survival</b>                |            |
| Median follow up, month (range)             | 9 months   |
| Median event free survival                  | 11 months  |
| 2 years                                     | 31.4%      |
| 3 years                                     | 28.4%      |
| 5 years                                     | 24.3%      |
| <b>Mortality rate</b>                       |            |
| Dead                                        | 63 (65.6%) |
| Alive                                       | 33 (34.4%) |

| Cause of death      |            |
|---------------------|------------|
| Disease progression | 41 (65.1%) |
| Infection           | 15 (23.8%) |
| Others              | 6 (9.5%)   |
| Unknown status      | 1 (1.6%)   |

#### 4.4 Characteristics of Transplanted Patients with B-ALL with *BCR-ABL1*

A total of 38 adult patients with female preponderance were identified (57.9%). The median age of transplant was 37.5 years old (range 14-69 years old). Three patients (7.9%) were in the adolescent age group of 12 to 19 years, while 19 patients (50%) were in the young adult age group of 20 to 39 years. The remaining 42.1% (n=16) were patients aged 40 years and older. The majority of patients were of Chinese ethnic group (47.4%, n=18) followed by Chinese (44.7%, n=17) and Indian (7.9%, n=3). 58.3% of the patients (n = 21) had a high white cell count ( $>30 \times 10^3 /\mu\text{L}$ ) at diagnosis. The median white cell count was  $36 \times 10^3 /\mu\text{L}$ . The characteristics of the patients who underwent transplantation are shown in Table 5.

For the transplant itself, the majority of patients (65.7%, n=25) were transplanted without ABO mismatch followed by minor mismatch (10.5%, n=4), major mismatch (5.2%, n=2) and bidirectional mismatch (5.2%, n=2). Among all patients who underwent stem cell transplantation, there were more patients in the M-M group (28.9%, n=11) followed by the F-F group (23.7%, n=9), Fe-M (21.1%, n=8) and M-F (7.9%, n=3). Of the 38 transplanted patients, 21 (55.3%) received allografts from the CMV positive donor and the CMV positive recipient, followed by allografts from the CMV negative donor to the CMV positive recipient (10.5%, n=4), while the least were



allografts from both CMV-negative donor and CMV-negative recipient, and the CMV positive donor to the CMV negative recipient (2.6%, n=1) (Table 5).

Table 5: Transplanted patients characteristics

| Baseline characteristics (n=38)    | N (%)          |
|------------------------------------|----------------|
| <b>Age, years</b>                  |                |
| Median (range)                     | 37.5 (14 - 69) |
| Mean                               | 37.05          |
| 12-19 (Adolescent)                 | 3 (7.9)        |
| 20- 39 (Young adult)               | 19 (50)        |
| 40- 59 (Adults)                    | 16 (42.1)      |
| ≥60 (Elderly)                      | 0 (0)          |
| <b>Ethnicity</b>                   |                |
| Malay                              | 17 (44.7)      |
| Chinese                            | 18 (47.4)      |
| Indian                             | 3 (7.9)        |
| Others                             | 0 (0)          |
| <b>Gender</b>                      |                |
| Male                               | 16 (42.1)      |
| Female                             | 22 (57.9)      |
| <b>WCC (<math>10^3</math> /uL)</b> |                |
| Median                             | 36             |
| Mean                               | 87             |
| >30                                | 21 (58.3)      |
| <30                                | 15 (41.7)      |
| <b>ABO mismatch</b>                |                |
| No mismatch                        | 25 (65.7)      |
| Minor mismatch                     | 4 (10.5)       |
| Major mismatch                     | 2 (5.2)        |
| Bidirectional mismatch             | 2 (5.2)        |

|                                            |           |
|--------------------------------------------|-----------|
| Missing                                    | 5 (13.2)  |
| <b>Gender mismatched (donor-recipient)</b> |           |
| Female to Male (F-M)                       | 8 (21.1)  |
| Male to Female (M-F)                       | 3 (7.9)   |
| Male to Male (M-M)                         | 11 (28.9) |
| Female to Female (F-F)                     | 9 (23.7)  |
| Missing                                    | 7 (18.4)  |
| <b>CMV status</b>                          |           |
| Both donor and recipient negative          | 1 (2.6)   |
| Both donor and recipient positive          | 21 (55.3) |
| Donor positive; Recipient negative         | 1 (2.6)   |
| Donor negative; Recipient positive         | 4 (10.5)  |
| Missing                                    | 11 (29.0) |

#### 4.5 Transplant Modalities and Outcome of Allogeneic Stem Cell Transplant

Transplant modalities include types of donors, stem cell source, conditioning, and GVHD prophylaxis (Table 6). The outcome of allogeneic stem cell transplantation is also shown in Table 6. The most common type of allogeneic HCT was transplantation of fully matched sibling donors (82.4%, n=145), followed by a non-related donor (8.5%, n=15) and a haplomatched related donor (2.8%, n=5). There was a small proportion of patients who underwent autologous HCT (4%, n=7). Peripheral blood stem cell (PBSC, n=167, 94.3%) was the main source of allograft followed by the marrow graft (3.4%, n=6) and the cord blood graft (2.3%, n=4). Most of the patients (96%, n=169) had undergone myeloablative conditioning chemotherapy. Only a small fraction of them (4%, n=7) had reduced intensity conditioning chemotherapy (RIC).

The TBI-based protocol was the main conditioning regimen (73%) and GVHD

prophylaxis included CSA/MTX (77.5%) and CSA/MMF (16.9%). Intravenous heparin (79.4%, n=135), low molecular weight heparin (17.6%, n=30) and defibrotide (2.9%, n=5) were used as prophylaxis of venoocclusive disease (VOD). The mean dose of stem cell dose infused were  $4.9 \times 10^6/\text{kg}$  with median TWBC and platelet engraftment of 13 days and 15 days, respectively. As shown in Table 4, the main causes of transplant-related death included infection (n=17), GVHD (n=8), lung toxicity (n = 3), transplant-related TMA (n = 1), venoocclusive disease (n=2), graft failure (n=1) and intracranial bleeding due to low platelet (n=1).

Table 6: Transplant modalities and outcomes in patients with B-ALL with *BCR-ABL1*

| Transplant modalities                                      | N (%)        |
|------------------------------------------------------------|--------------|
| <b>Types of transplants</b>                                |              |
| Matched Allogenic sibling                                  | 28 (73.7)    |
| Matched unrelated donor                                    | 3 (7.9)      |
| Haplo-matched                                              | 1 (2.6)      |
| Cord blood                                                 | 1 (2.6)      |
| Autologous                                                 | 5 (13.2)     |
| <b>Stem cell source</b>                                    |              |
| PBSC                                                       | 37 (97.4)    |
| BM                                                         | 0            |
| Umbilical cord                                             | 1 (2.6)      |
| <b>Stem cell dose (<math>\times 10^6/\text{kg}</math>)</b> |              |
| Median                                                     | 4.9 (3.0-11) |
| <b>Conditioning regime</b>                                 |              |



|                                             |           |
|---------------------------------------------|-----------|
| Myeloablative                               | 34 (89.5) |
| Reduced intensity chemotherapy              | 4 (10.5)  |
| TBI based                                   | 25 (65.8) |
| Non TBI                                     | 13 (34.2) |
| <b>GVHD prophylaxis</b>                     |           |
| CSA/MTX                                     | 25 (65.8) |
| CSA/MMF                                     | 5 (13.2)  |
| CSA/MTX/ATG                                 | 2 (5.3)   |
| Missing                                     | 6 (15.8)  |
| <b>VOD Prophylaxis</b>                      |           |
| IV heparin                                  | 26 (68.4) |
| LMWH                                        | 10 (26.3) |
| Defibrotide                                 | 2 (5.3)   |
| <b>Remission status prior to transplant</b> |           |
| CR1                                         | 32 (84.2) |
| CR>1                                        | 6 (15.8)  |
| <b>Engraftment (days)</b>                   |           |
| Median time for WCC (9-25)                  | 13        |
| Median time for platelet (8-70)             | 15        |
| <b>Post-transplant response</b>             |           |
| Remission                                   | 30 (78.9) |
| Relapsed                                    | 8 (21.1)  |
| <b>GVHD</b>                                 |           |

|                                             |           |
|---------------------------------------------|-----------|
| Acute                                       | 11 (28.9) |
| No GVHD                                     | 18 (47.4) |
| Grade I                                     | 2         |
| Grade II                                    | 3         |
| Grade III                                   | 4         |
| Grade IV                                    | 2         |
| Missing                                     | 9         |
| Chronic                                     | 9 (23.7)  |
| Limited                                     | 3         |
| Extensive                                   | 6         |
| <b>Molecular BCR ABL achieving MMR</b>      |           |
| <b>Post end of treatment/pre-transplant</b> |           |
| ≥0.1%                                       | 8 (21.1)  |
| <0.1%                                       | 26 (68.4) |
| NA                                          | 3 (10.5)  |
| <b>Post-transplant 3 months</b>             |           |
| ≥ 0.1%                                      | -         |
| < 0.1%                                      | 21 (55.3) |
| NA                                          | 17 (44.7) |
| <b>Overall survival</b>                     |           |
| Median follow up, month (2-228)             | 15 months |
| Median overall survival                     | 63 months |
| 2 years                                     | 57.6%     |
| 3 years                                     | 52.3%     |
| 5 years                                     | 51.1%     |
| <b>Disease Free Survival</b>                |           |
| Median follow up, month (range)             | 15 months |

|                              |           |
|------------------------------|-----------|
| Median event free survival   | 60 months |
| 2 years                      | 62.1%     |
| 3 years                      | 56.4%     |
| 5 years                      | 49.4%     |
| <b>Mortality rate</b>        |           |
| Dead                         | 16 (42.1) |
| Alive                        | 22 (57.9) |
| <b>Cause of death (n=16)</b> |           |
| Disease progression          | 8 (50.0%) |
| Infection                    | 5 (31.3%) |
| Others                       | 3 (7.9%)  |
| VOD                          | 1         |
| GVHD                         | 2         |

#### 4.7 Patients Achieving CMR Using *BCR-ABL1* Transcript Levels at Different Time Points

A total of 33 patients (34.4%) who had completed chemotherapy were transplant eligible and underwent allogeneic stem cell transplantation while 5 patients (13.2%) underwent autologous transplant. The median age of patients receiving allogeneic transplant was 37 years (range 15-59 years). All patients received allografts, with the majority being a matched-sibling donor (n=27) (Table 6). Most of the patients (87.9%, n=29) underwent myeloablative conditioning chemotherapy with the TBI-based protocol being the main conditioning regimen (66.7%), where 13 (44.8%) developed acute graft-versus-host disease (aGVHD) while 14 (50.0%) developed chronic GVHD (cGVHD).

The univariate analysis of the predictive factors for OS is shown in Table 7. Only MRD status at TP3 and patients achieving CR1 were clinically significant. Multivariate analysis showed that the high-risk group and transplanted in CR>1 were independent prognostic factors for inferior OS (*BCR-ABL1* level pre-transplant: HR 4.358,  $p=0.017$ ; Transplant in CR>1: HR 4.582,  $p=0.016$ ) and DFS (*BCR-ABL1* level pre-transplant: HR 4.106,  $p=0.002$ ; transplant in CR>1: HR 3.787,  $p=0.033$ ) shown in Table 8, Figure 4 and Figure 5. MRD status by molecular quantitation at pre-transplant had better distinction for OS ( $p=0.005$ ) and DFS ( $p=0.002$ ) compared to post-induction ( $p=0.70$ ,  $p=0.75$ ) and post-consolidation ( $p=0.138$ ,  $p=0.078$ ) respectively (Figure 4 and Figure 5). With a median follow-up of 25 months, the median overall survival month was not reached. The 2-year, 3-year and 5-year OS rates were 66.3%, 58.7% and 53.4%, while the 2-year, 3-year and 5-year DFS rates were 63.7%, 59.9% and 54.5% (Figure 3D), respectively. With a median follow-up of 9 months, the median DFS month was not reached. At the time of analysis, 20 patients were alive (60.6%) while 13 had died (39.4%) as shown in Table 10. Six (46.2%) of 13 patients with MMR experienced relapse with a median time to relapse of 14 months (range 8 to 104 months).

Table 7: Univariable analysis of factors predictive for OS

| Characteristic               | B-ALL with <i>BCR-ABL1</i> |        | Transplanted B-ALL with <i>BCR-ABL1</i> |       |  |      |
|------------------------------|----------------------------|--------|-----------------------------------------|-------|--|------|
|                              | 3-year OS (%)              | p      | 3-year OS (%)                           | p     |  |      |
| Age, years                   |                            |        |                                         |       |  |      |
| AYA                          | 39.0                       | 0.064  | 52.9                                    | 0.690 |  |      |
| Adult and elderly            | 19.6                       |        | 55.0                                    |       |  |      |
| Gender                       |                            |        |                                         |       |  |      |
| Male                         | 33.6                       | 0.876  | 54.2                                    | 0.906 |  |      |
| Female                       | 26.8                       |        | 52.9                                    |       |  |      |
| WCC at diagnosis             |                            |        |                                         |       |  |      |
| >30x10 <sup>3</sup> /μL      | 25.5                       | 0.585  | 52.3                                    | 0.684 |  |      |
| <30 x10 <sup>3</sup> /μL     | 35.9                       |        | 63.6                                    |       |  |      |
| MRD status (%)               | ≥0.1                       | <0.1   | ≥0.1                                    | <0.1  |  |      |
| TP1                          | 36.7                       | 36.1   | 0.974                                   |       |  |      |
| TP2                          | 23.5                       | 53.1   | 0.077                                   |       |  |      |
| TP3                          | 11.5                       | 65.4   | 0.005                                   |       |  | 25.0 |
| Chemotherapy                 |                            |        |                                         |       |  |      |
| GMALL/BFM                    | 25.7                       | 0.081  |                                         |       |  |      |
| Hyper-CVAD                   | 33.7                       |        |                                         |       |  |      |
| TKI                          |                            |        |                                         |       |  |      |
| Yes (2012-2018)              | 33.7                       | 0.390  |                                         |       |  |      |
| No (2006-2011)               | 23.5                       |        |                                         |       |  |      |
| Transplant                   |                            |        |                                         |       |  |      |
| Yes                          | 58.9                       | <0.001 |                                         |       |  |      |
| No                           | 10.6                       |        |                                         |       |  |      |
| Disease status at transplant |                            |        |                                         |       |  |      |
| CR1                          |                            |        | 65.9                                    | 0.003 |  |      |
| >CR1                         |                            |        | 16.7                                    |       |  |      |

Values are presented as number (%). Significant *p*-value <0.05. AYA, adolescent and young adult; WCC, white cell count; TP1, Time-point 1; TP2, Time-point 2; TP3,



Time-point 3; MRD, measurable residual disease; TKI, tyrosine kinase inhibitor; CR, complete remission.

Table 8: Multivariable analysis of factors predictive of OS and DFS in transplanted patients.

| Characteristic                                               | Risk of relapse or death |                |       | Risk of death |                |       |
|--------------------------------------------------------------|--------------------------|----------------|-------|---------------|----------------|-------|
|                                                              | HR                       | 95% CI         | p     | HR            | 95% CI         | p     |
| <b>BCR-ABL1 level pre-transplant <math>\geq 0.1\%</math></b> | 4.106                    | 1.226 – 13.752 | 0.022 | 4.358         | 1.301 – 14.597 | 0.017 |
| <b>Transplant in CR&gt;1</b>                                 | 3.787                    | 1.113 – 11.520 | 0.033 | 4.582         | 1.352 – 15.528 | 0.016 |

CR, complete remission; HR, hazard ratio;  $p$ -value  $< 0.05$ .

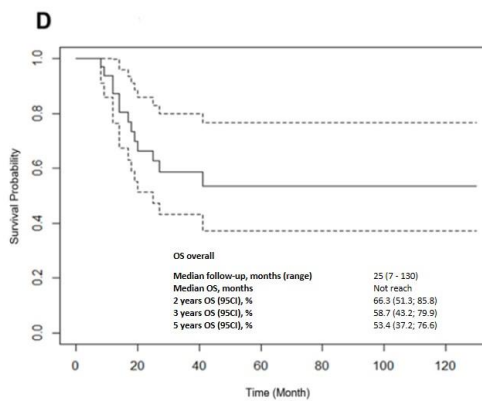
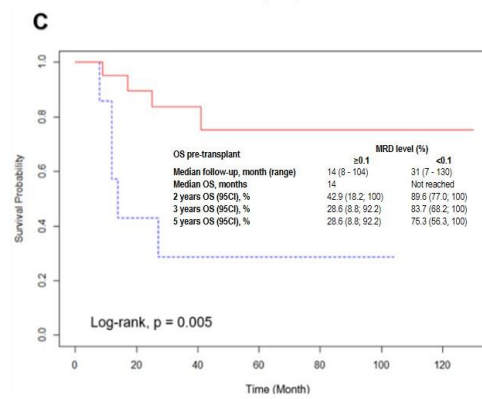
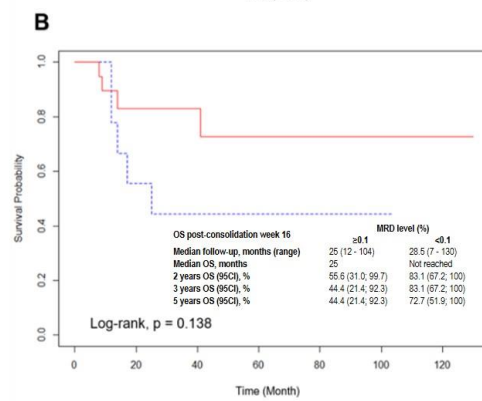
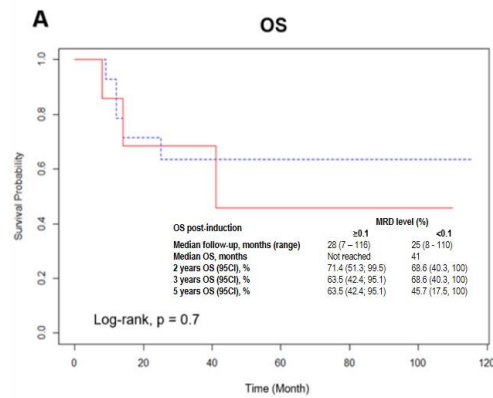


Figure 4: Transplantation outcome, OS according to *BCR-ABL1* at certain time points. (A) Post-induction MRD (B) Post-consolidation week 16 (C) End of treatment (D) The overall cohort



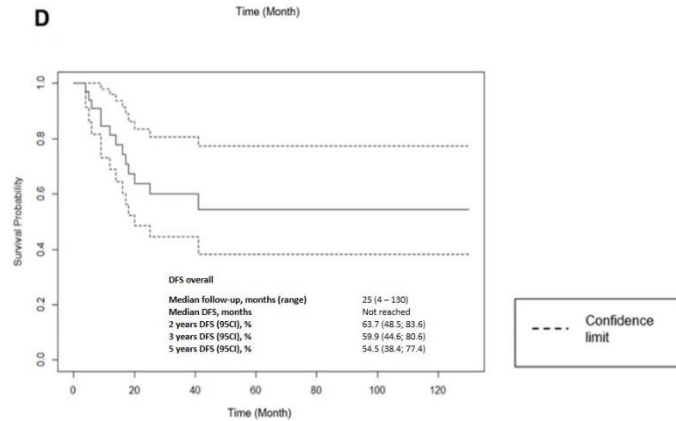
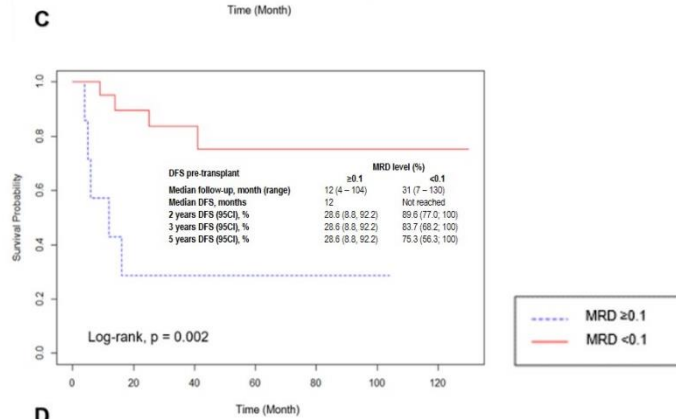
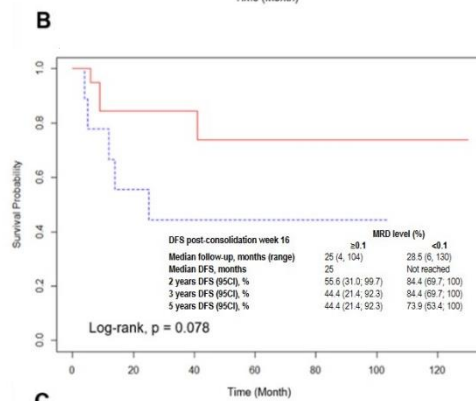
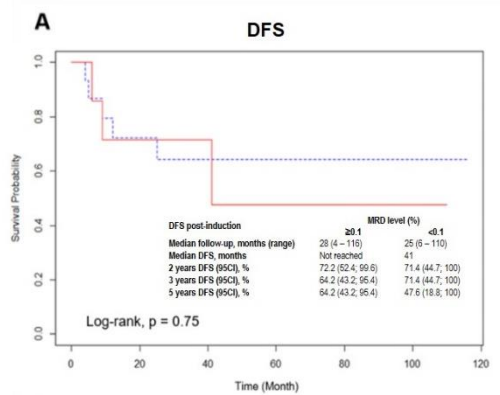


Figure 5: Transplantation outcome, DFS according to *BCR-ABL1* at certain time points. (A) Post-induction MRD (B) Post-consolidation week 16 (C) End of treatment (D) The overall cohort

