Supplemental Data

Copy number alterations in B-cell development genes, drug resistance, and clinical outcome in pediatric B-cell precursor acute lymphoblastic leukemia

Elisabeth M.P. Steeghs[1], Judith M. Boer[1,2], Alex Q. Hoogkamer[1], Aurélie Boeree[1], Valerie de Haas[3], Hester A. de Groot-Kruseman[3], Martin A. Horstmann[4], Gabriele Escherich[4], Rob Pieters[2,3], and Monique L. den Boer[1,2,3,*]

[1] Department of Pediatric Oncology/Hematology, Erasmus Medical Center – Sophia Children's Hospital, Rotterdam, the Netherlands

[2] Princess Máxima Center for Pediatric Oncology, Utrecht, The Netherlands.

[3] DCOG, Dutch Childhood Oncology Group, The Hague, The Netherlands

[4] COALL - German Cooperative Study Group for Childhood Acute Lymphoblastic Leukemia, University Medical Centre Eppendorf, Martinistrasse 52, 20246 Hamburg, Germany

Running short title: CNAs in B-cell development genes in pediatric ALL

Correspondence:

Monique L. den Boer, PhD

Princess Máxima Center for Pediatric Oncology

P.O. Box 113

NL-3720 AC Bilthoven

Phone: +31 88 97 272 72

Supplementary Table 1: Genomic lesions in *BCR-ABL1*-like and B-other cases

	***BCR-ABL1*-like**			**B-other**			***BCR-ABL1*-like and B-other**		
	***IKZF1* del**	**No *IKZF1* del**	**p-value**	***IKZF1* del**	**No *IKZF1* del**	**p-value**	***IKZF1* del**	**No *IKZF1* del**	**p-value**
Tyrosine kinase fusion	24.2% (8/33)	12.2% (5/41)	0.23	0% (0/13)	0% (0/58)	1	17.4% (8/46)	5.1% (5/99)	**0.026**
***ERG* deletion**	0% (0/31)	0% (0/41)	1	4.2% (1/24)	14.0% (8/57)	0.268	1.8% (1/55)	8.1% (8/99)	0.16
Dic(9;20)	34.2% (12/35)	14.6% (7/48)	0.06	3.8% (1/26)	2.9% (2/68)	1	21.3% (13/61)	7.8% (9/116)	**0.015**
iAMP21	8.6% (3/35)	18.8% (9/48)	0.23	3.8% (1/26)	1.5% (1/68)	0.479	6.6% (4/61)	8.6% (10/116)	0.77
Chromosome 9p deletion	40% (14/35)	25% (12/48)	0.16	19.2% (5/26)	16.2% (11/68)	0.763	31.1% (19/61)	19.8% (23/116)	0.10

Supplementary Table 2: Clinical characteristics of the DCOG-ALL10 cohort

Clinical Features		**DCOG-ALL10 n=210**
Male		108/210 (51%)
age ≥ 10 years		37/210 (18%)
WBC ≥ 50x10^9/L		25/207 (12%)
Risk arm MR or HR		139/201 (69%)
Prednisone poor response[a]		11/203 (5%)
No CR after induction[b]		2/199 (1%)
NCI high risk[c]		65/209 (31%)
MRD TP1[d]	high	39/181 (22%)
	intermediate	12/181 (7%)
	low	130/181 (72%)
MRD TP2[d]	high	7/181 (4%)
	intermediate	6/181 (3%)
	low	168/181 (93%)

[a] Prednisone response on day 8 ≥1.0x10^9 blasts/L (DCOG ALL-10 protocols).
[b] Complete remission (CR) after induction (day 33 in DCOG ALL-10) is defined on morphological grounds by the presence of <5% leukemic blasts and regenerating hematopoiesis.
[c] NCI-Rome high risk is defined by white blood cell count (WBC) ≥_50x10^9/L and/or age ≥ 10 years.
[d] Minimal residual disease PCR high (≥10^{-3}), intermediate (≥10^{-4} and <10^{-3}), low (<10^{-4}); TP1, after the first induction course of chemotherapy, day 33; TP2, before consolidation, day 79.

Supplementary Figure Legends

Supplementary Figure 1. Overview of cohort screened for CNAs.

Flowchart showing the number of cases tested for CNAs in *IKZF1*, *EBF1*, *PAX5*, *ETV6*, *CDKN2A/B*, *RB1*, *BTG1*, and the PAR1 region. Cells from a selection of cases were used to study the drug sensitivity. The prognostic relevance of the CNAs (i.e. long-term prognosis and MRD levels) were studied in DCOG-ALL10 cases.

Supplementary Figure 2. CNA landscape of B-cell development genes in the different subtypes of pediatric BCP-ALL.

CNA profile of 515 newly diagnosed pediatric BCP-ALL patients, representing the major BCP-ALL subtypes, was determined using MLPA.

(**A**) The frequency of any CNA (*IKZF1*, *EBF1*, *PAX5*, *CDKN2A/B*, *RB1*, *BTG1*, *ETV6*, and/or PAR1) is shown per subtype. One CNA was sufficient to be quantified as altered. **p≤0.01

(**B**) The association between *IKZF1* deletions and BCP-ALL subtypes. *IKZF1* deletions are depicted as deletion of exon 4-7, deletions of exon 1-8, or remaining deletions.

Supplementary Figure 3. The association between CNAs and MRD levels after induction therapy and the first consolidation course in *BRC-ABL1*-like and B-other patients.

MRD levels of DCOG-ALL treated *BCR-ABL1*-like and non-*BCR-ABL1*-like B-other cases after induction (TP1; n=47) and first consolidation course (TP2; n=46). The percentage of cases with high ($\geq 10^{-3}$), medium ($10^{-3} > MRD \geq 10^{-4}$), and undetectable MRD levels ($<10^{-4}$) is depicted per CNA.

Fisher's Exact test. **p≤0.01, *p≤0.05. del = deletion.

Supplementary Figure 4. The association between CNAs and MRD levels after induction therapy and the first consolidation course in high hyperdiploid patients.

MRD levels of DCOG-ALL treated high hyperdiploid cases after induction (TP1; n=60) and first consolidation course (TP2; n=59). The percentage of cases with high ($\geq 10^{-3}$), medium ($10^{-3} > MRD \geq 10^{-4}$), and undetectable MRD levels ($<10^{-4}$) is depicted per CNA.

Fisher's Exact test. **p≤0.01, *p≤0.05. del = deletion.

Supplementary Figure 5. The association between CNAs and MRD levels after induction therapy and the first consolidation course in *ETV6-RUNX1* patients.

MRD levels of DCOG-ALL treated *ETV6-RUNX1* cases after induction (TP1; n=65) and first consolidation course (TP2; n=66). The percentage of cases with high (≥10^{-3}), medium (10^{-3} > MRD ≥ 10^{-4}), and undetectable MRD levels (<10^{-4}) is depicted per CNA.

Fisher's Exact test. **p≤0.01, *p≤0.05. del = deletion.

Supplementary Figure 6. The association between any CNA and MRD levels after induction therapy and the first consolidation course.

MRD levels of DCOG-ALL treated BCP-ALL cases after induction (TP1; n=183) and first consolidation course (TP2; n=183). The MRD levels of cases with any or no CNA are depicted for all subtypes, *BCR-ABL1*-like and non-*BCR-ABL1*-like B-other, high hyperdiploid, and *ETV6-RUNX1*.

Supplementary Figure 7. Prognostic value of CNAs in DCOG-ALL10 treated cases.

The association between CNAs and cumulative incidence of relapse (CIR) or event-free-survival (EFS) was examined. Patients were treated according to DCOG-ALL10 protocol. CIR was estimated using a competing risk model. Relapse and non-response were considered as event, and death as competing event. To test equality of the CIRs, the Gray's test was applied. Non-response, relapse, and death were considered as events for EFS. EFS rates were determined using Cox regression, and compared using the Wald test.

(**A**) CIR and EFS curves of cases without or with a *RB1* deletion. Curves contain either all risk groups, or the medium risk arm only.

(**B**) CIR and EFS curves of *BCR-ABL1*-like and non-*BCR-ABL1*-like B-other cases without or with *BTG1* deletions.

Supplementary Figure 8. Cellular drug response and CNAs

Leukemic cells were incubated for four days with an increasing concentration range of prednisolone (µg/ml), vincristine (µg/ml), L-asparaginase (IU/ml), daunorubicin (µg/ml), 6-mercaptopurine (µg/ml), and 6-thioguanine (µg/ml), after which cell viability was measured using an MTT assay. Association between indicated CNAs and *ex vivo* cytotoxicity was studied. The Mann-Whitney U test was applied to compare LC50-values. * $p < 0.05$, ** $p < 0.01$

(**A**) LC50 values of L-asparaginase (IU/ml) of primary leukemic cells without or with *IKZF1* deletion. Columns include all BCP-ALL subtypes (grey), BCR-ABL1-like/ B-other cells (blue), and high hyperdiploid cells (green). The red line represent the median LC50 value in the each group.

(**B**) LC50 values of prednisolone (µg/ml) of primary leukemic cells without an *IKZF1* or *PAX5* CNA, with *IKZF1* and *PAX5* CNA, a *PAX5* CNA without an *IKZF1* del, or *IKZF1* an del without a *PAX5* CNA. The red and black lines represent the median LC50 value in the each group.

(**C**) LC50 values of L-asparaginase (IU/ml) and 6-thiogunanine (µg/ml) of primary leukemic cells without or with *ETV6* deletion. Columns include all BCP-ALL subtypes (grey), BCR-ABL1-like/ B-other cells (blue), high hyperdiploid cells (green), and *ETV6-RUNX1*-positive cells (yellow). The red line represent the median LC50 value in the each group.

Supplementary Figure 9: The associations between the *IKZF1*^{plus}, long-term prognosis, MRD levels, and cellular drug resistance.

(**A**) Cumulative incidence of relapse and event-free survival curves are shown for patient enrolled in the DCOG-ALL10 protocol. The p-values of the curves of *IKZF1*^{plus}, *IKZF1*^{del} only, and *IKZF1*^{del} groups compared to the *IKZF1* wildtype group are reported. The dotted line (*IKZF1*^{del}) represents the total group of *IKZF1*^{plus} and *IKZF1*^{del} only cases (n=30). (**B**) MRD levels of DCOG-ALL10 treated BCP-ALL cases (all risk groups) after induction (TP1; n=183) and first consolidation course (TP2; n=183). The percentage of cases with high (≥10-3), medium (10 4 ≤ MRD < 10 3), and low MRD levels (<10-4) is depicted. (**C**) Leukemic cells were incubated for four days with a concentration range of prednisolone (µg/ml), vincristine (µg/ml), L-asparaginase (IU/ml), daunorubicin (µg/ml), 6-mercaptopurine (µg/ml), and 6 thioguanine (µg/ml), after which cell viability was measured using an MTT assay. LC50 values are

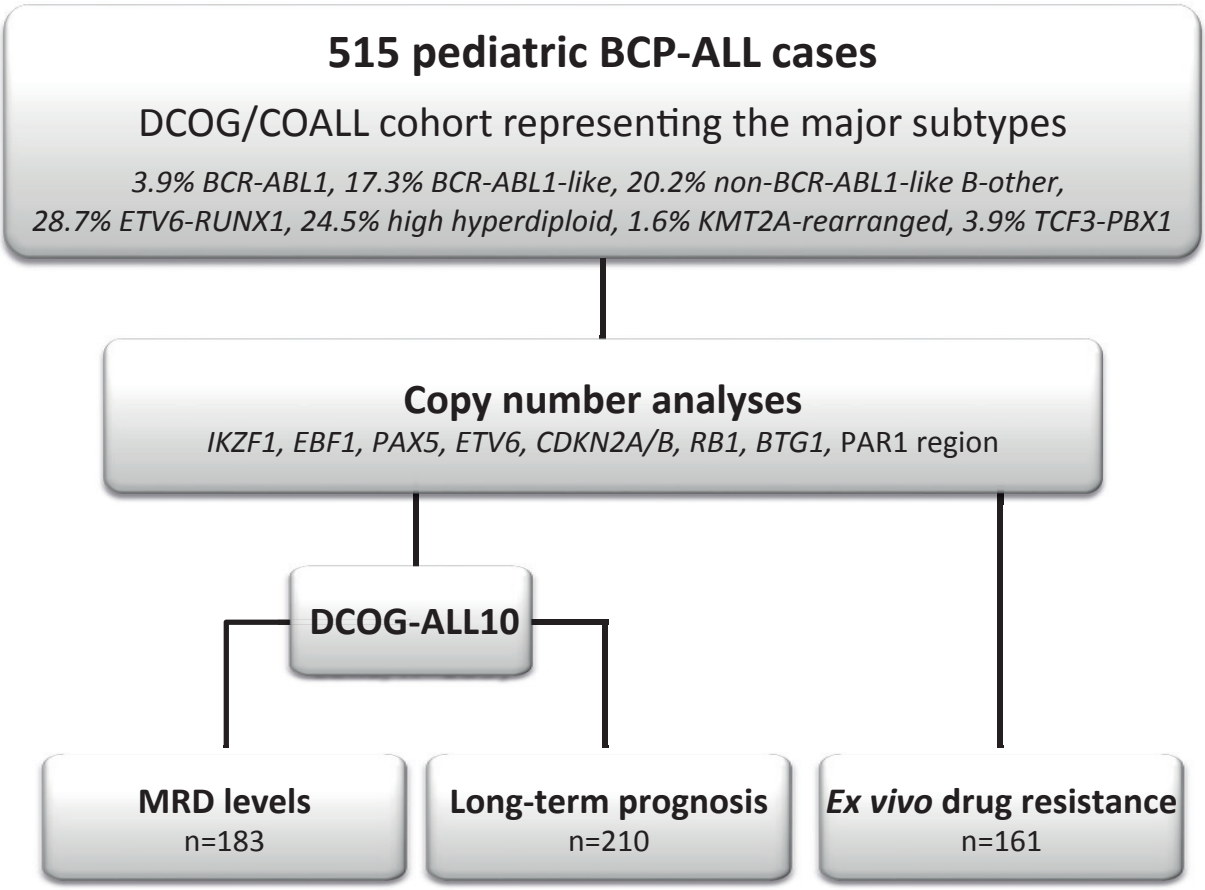
depicted. The red line represent the median LC50 value in the each group. * $p < 0.05$, ** $p < 0.01$; p-values against the IKZF1 wildtype group are reported.

Note: *IKZF1*^{plus}: presence of an *IKZF1*^{del} combined with loss of *PAX5* and/or *CDKN2A/B* (loss of PAR1 was not observed in this group). *IKZF1*^{del} only: cases with an *IKZF1* deletion that do not fulfill the *IKZF1*^{plus} criteria; *IKZF1*^{del}: presence of an *IKZF1* deletion involving both *IKZF1*^{plus} and *IKZF1*^{del} only cases.

Supplementary Figure 10: Integrated cytogenetic and genomic risk stratification in the DCOG-ALL10 cohort.

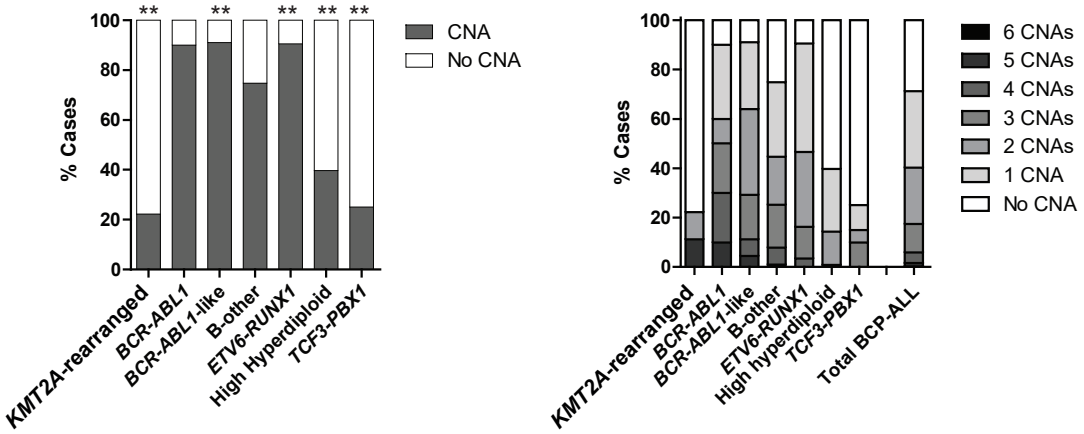
Cases were classified based on the risk stratification described by Moorman *et* al[1], which incorporates cytogenetic and CNA data. (**A**) Cumulative incidence of relapse and event-free survival curves of the UK good risk score and UK poor risk score are shown. (**B**) MRD levels of DCOG-ALL10 treated BCP-ALL cases after induction (TP1; n=183) and first consolidation course (TP2; n=183). The percentage of cases with high ($\geq 10^{-3}$), medium ($10^{-4} \leq MRD < 10^{-3}$), and undetectable MRD levels ($<10^{-4}$) is depicted. (**C**) Leukemic cells were incubated for four days with a concentration range of prednisolone (µg/ml), vincristine (µg/ml), L-asparaginase (IU/ml), daunorubicin (µg/ml), 6-mercaptopurine (µg/ml), and 6-thioguanine (µg/ml), after which cell viability was measured using an MTT assay. LC50 values are depicted. The red line represents the median LC50 value in each group. * $p < 0.05$, ** $p < 0.01$

Supplementary Figure 1. Overview of cohort screened for CNAs, cellular drug resistance, and clinical outcome parameters.



Supplementary Figure 2. CNA landscape of B-cell development genes in the different subtypes of pediatric BCP ALL.

A



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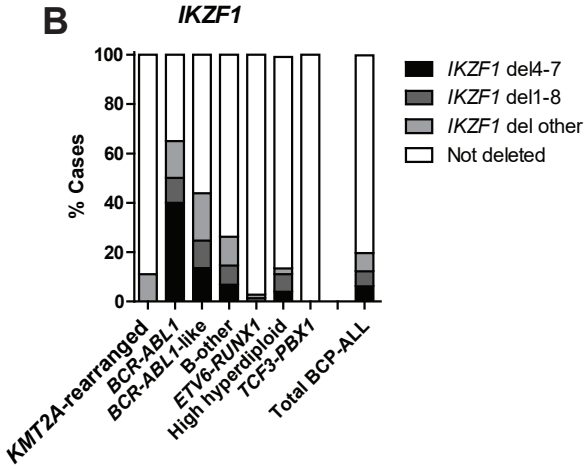
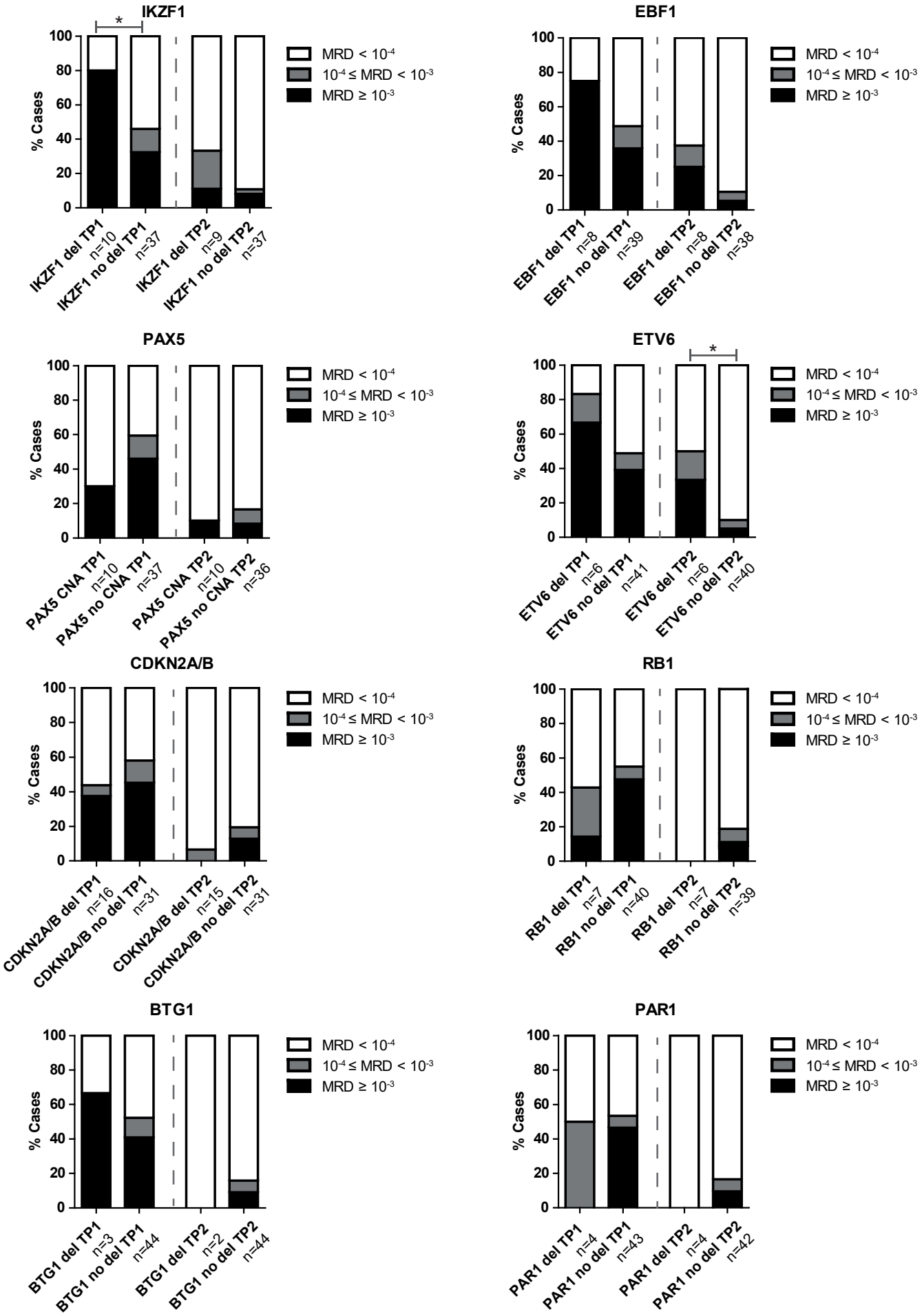


Supplementary Figure 3. The association between CNAs and MRD levels after induction therapy and the first consolidation course in *BRC-ABL1*-like and B-other patients.



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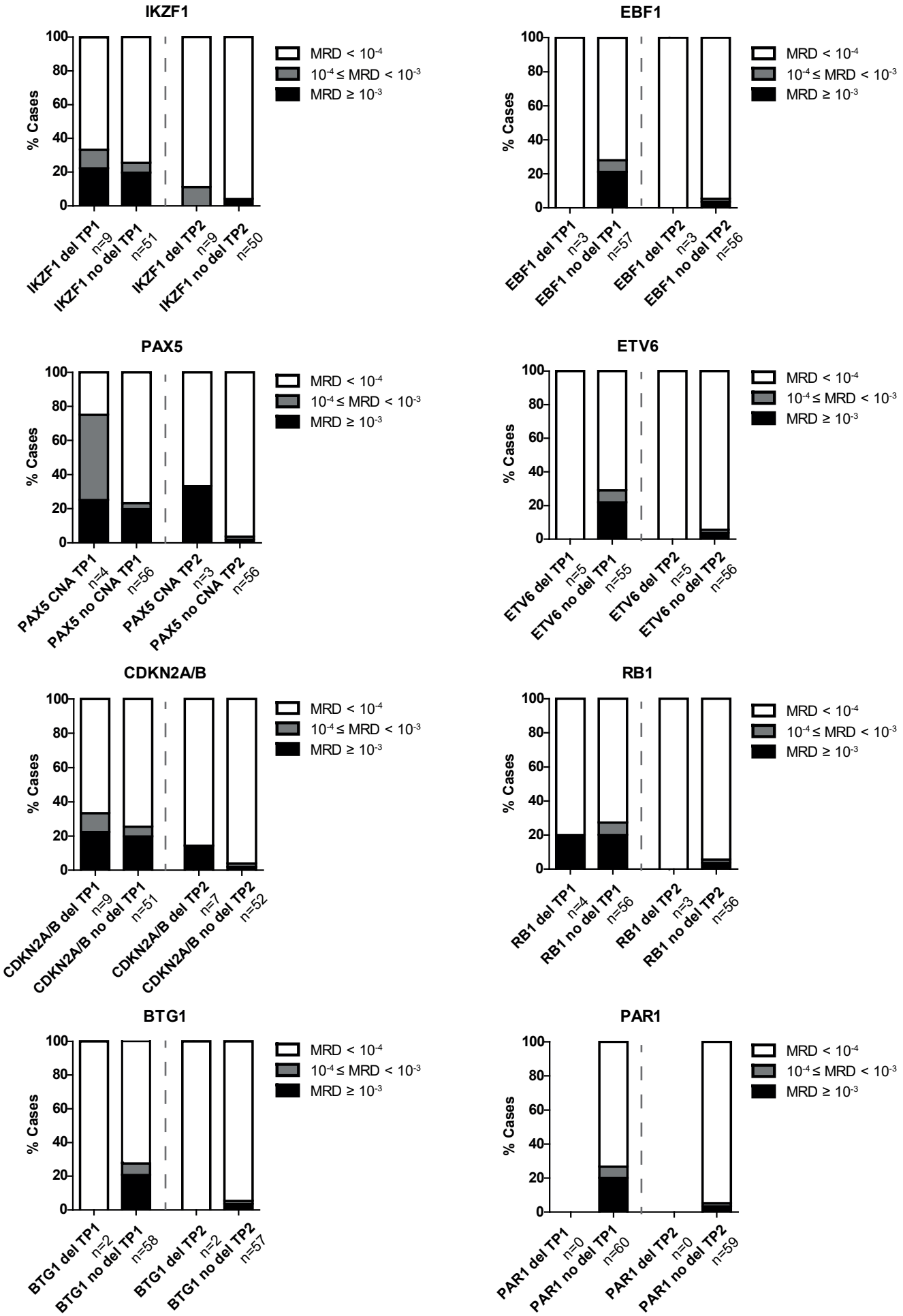

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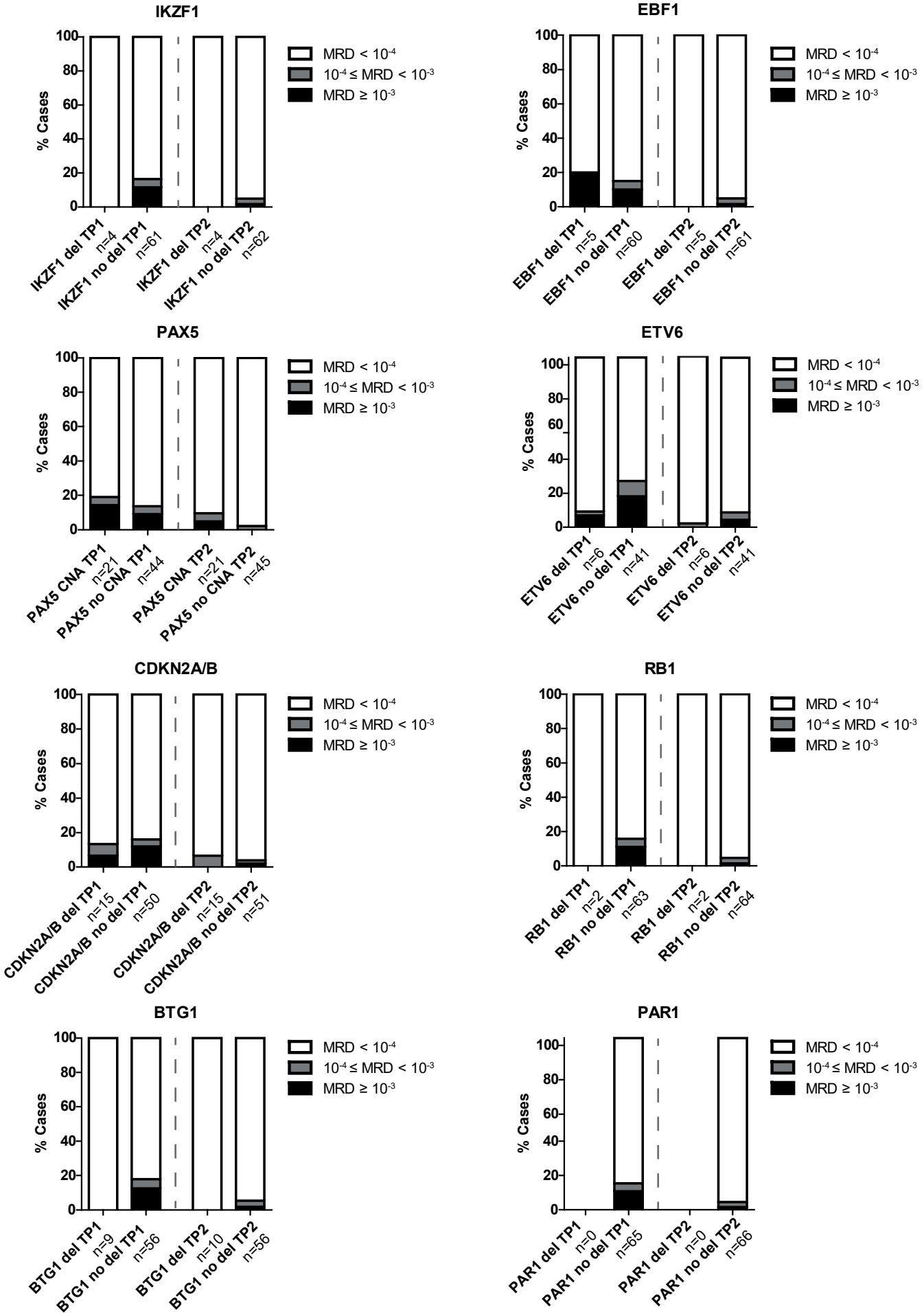
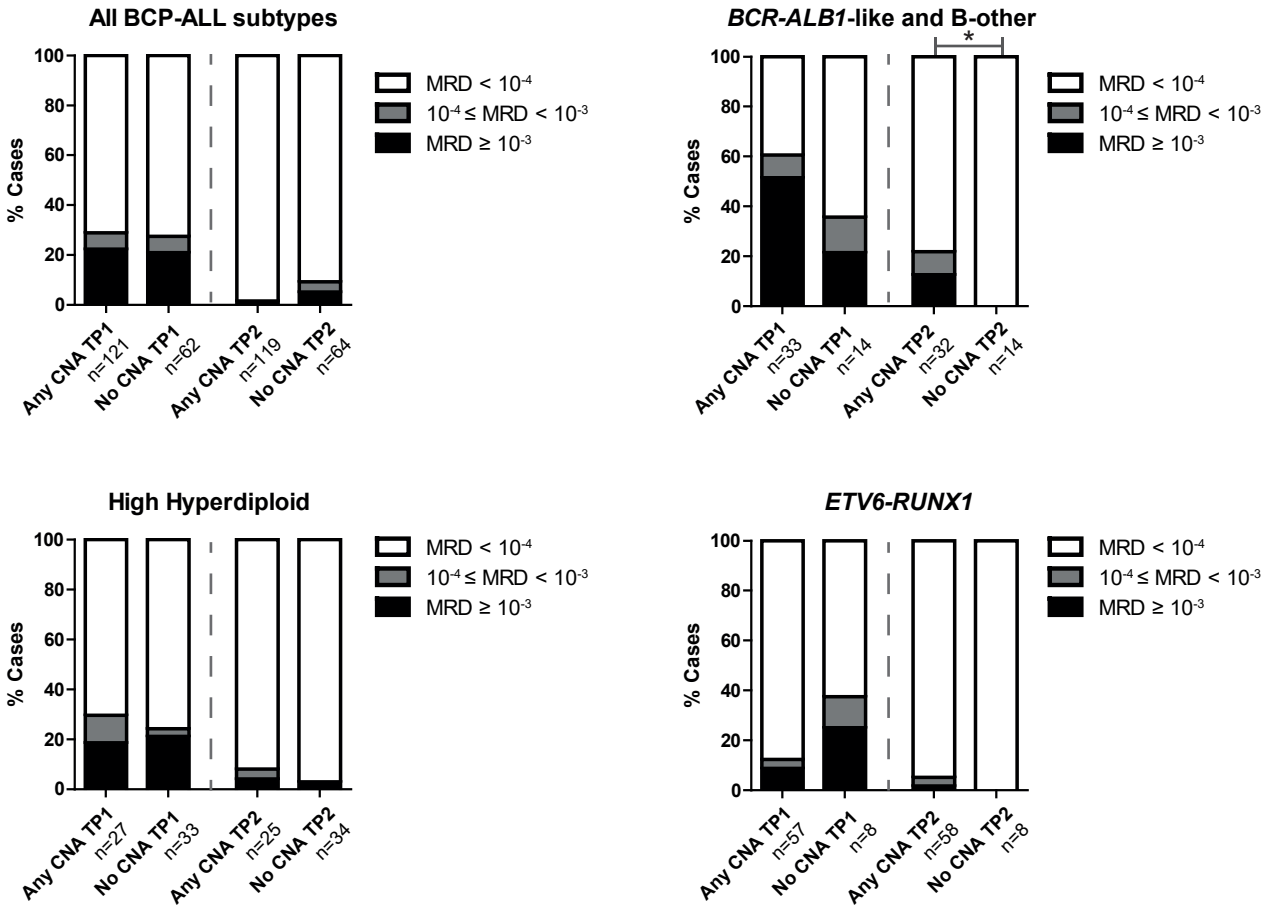
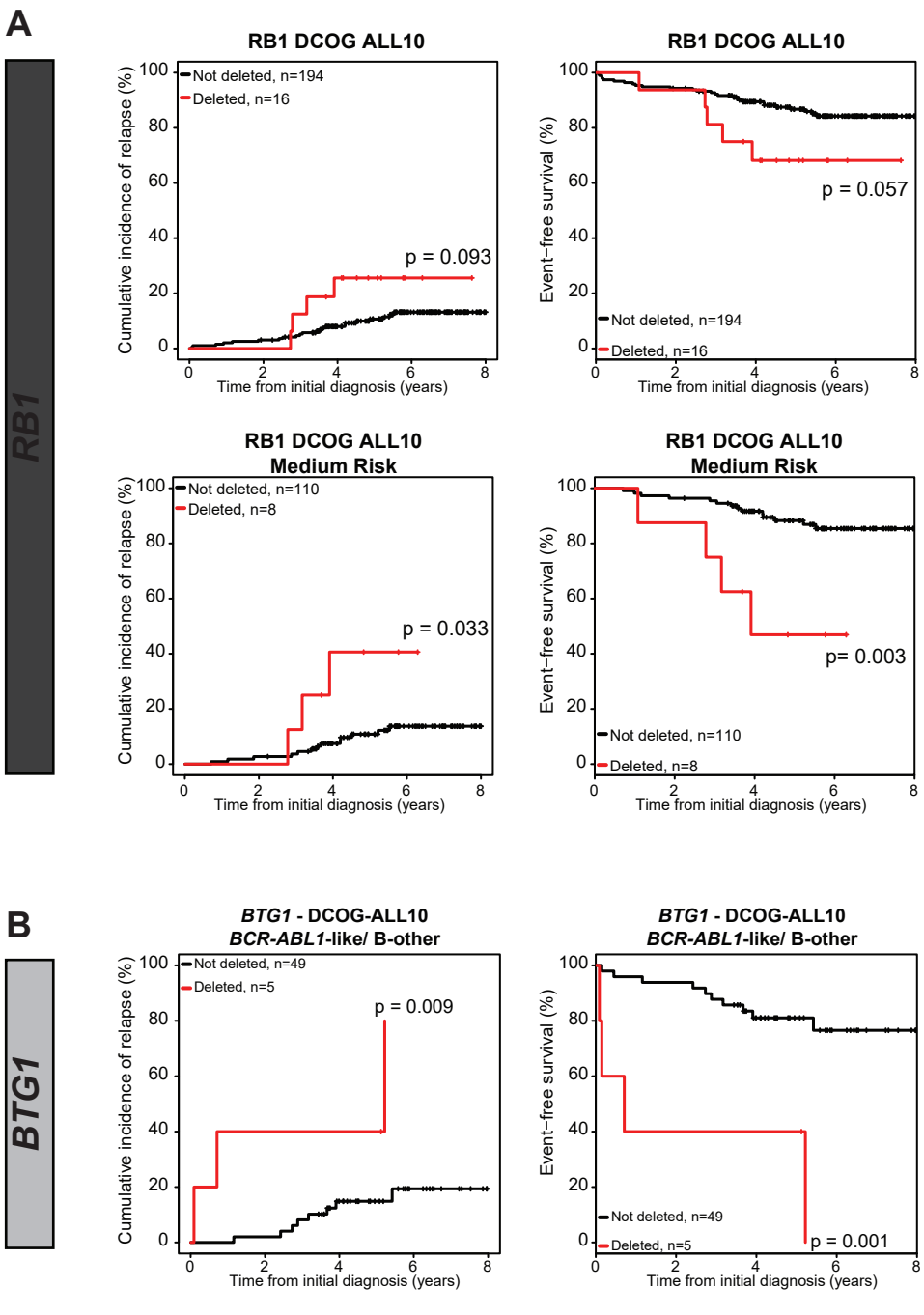


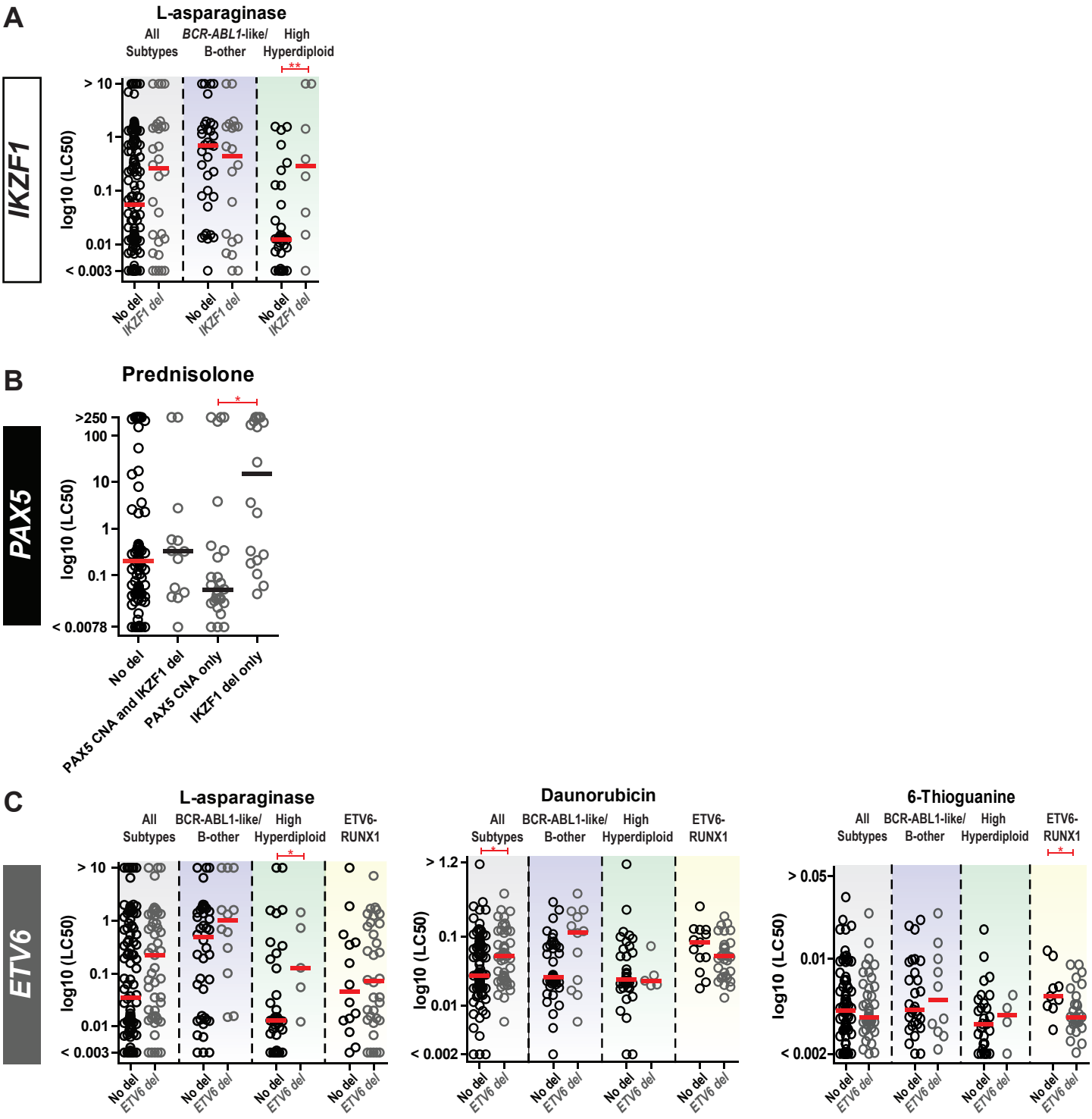
Supplementary Figure 6. The association between any CNA and MRD levels after induction therapy and the first consolidation course.



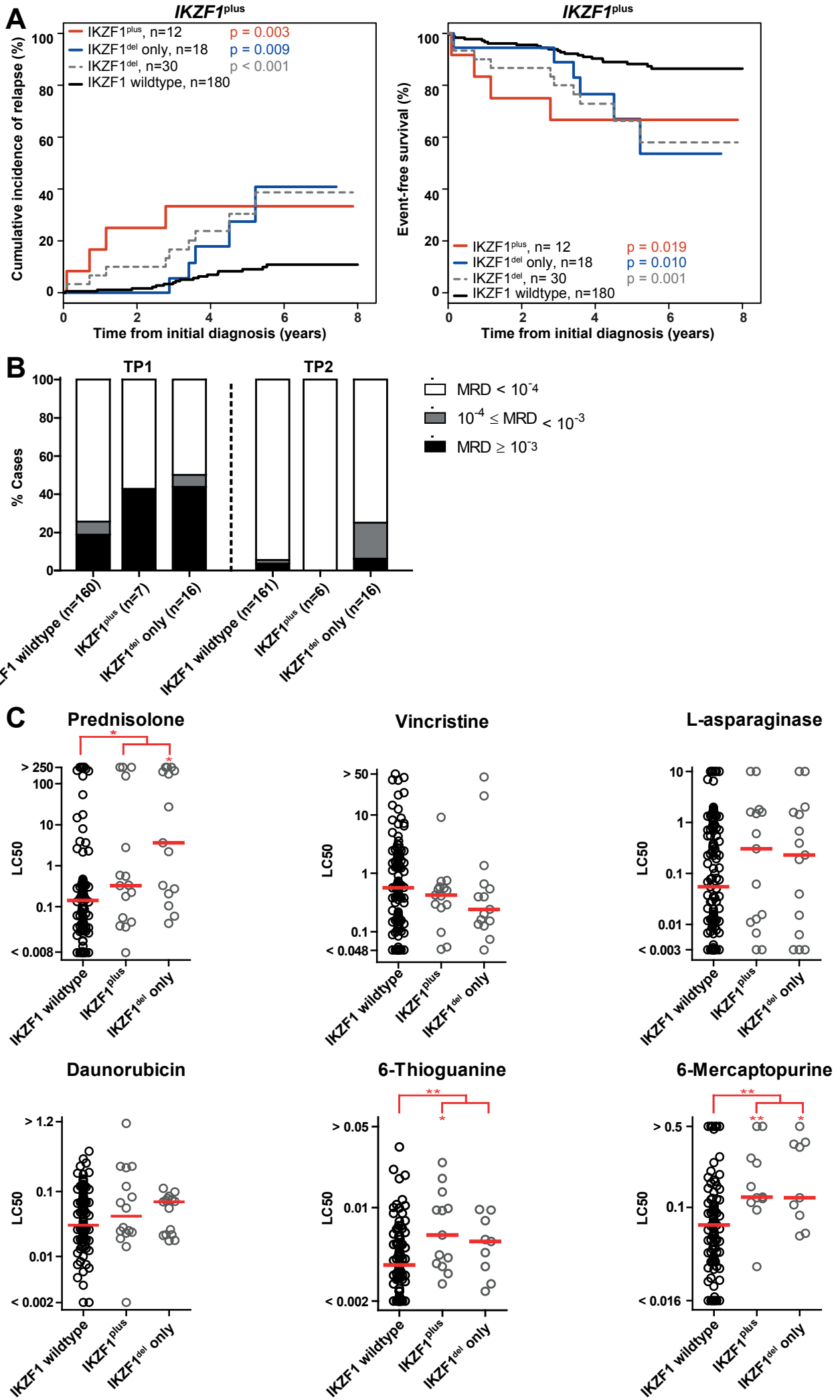
Supplementary Figure 7. Prognostic value of CNAs in DCOG-ALL10 treated cases.



Supplementary Figure 8. The association between CNAs and the *ex vivo* cellular drug response.



Supplementary Figure 9. *IKZF1*^{plus} classification: prognostic relevance and cellular drug resistance.



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