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Drugging DNA repair to target T-ALL cells

Yashodhara Dasgupta[a], **Konstantin Golovine**[a], **Margaret Nieborowska-Skorska**[a], **Li Luo**[b], **Ksenia Matlawska-Wasowska**[c], **Charles G. Mullighan**[d], and **Tomasz Skorski**[a]

[a]Department of Microbiology and Immunology, Fels Institute for Cancer Research & Molecular Biology, Temple University Lewis Katz School of Medicine, Philadelphia, PA, USA

[b]Division of Epidemiology, Biostatistics and Preventive Medicine, Department of Internal Medicine, University of New Mexico, Albuquerque, NM, USA

[c]Division of Pediatric Research, Department of Pediatrics, University of New Mexico Health Sciences Center, Albuquerque, NM, USA

[d]Department of Pathology, St. Jude Children's Research Hospital, Memphis, TN, USA

Despite recent progress in treatment, prognostication for T-cell acute lymphoblastic leukemia (T-ALL) patients, including those with early T-cell precursor ALL (ETP-ALL), is rather poor [1]. Therefore, new therapies are needed to extend the complete remission time/cure and/or to treat refractory T-ALL/ETP-ALL patients.

T-ALL accumulate potentially lethal 'spontaneous' DNA double-strand breaks (DSBs) caused by RAG1/2 in ATM-deficient cells and/or by reactive oxygen species (ROS) [2,3]. In addition, one of the goals of intensive therapies is to induce DSBs. Thus, T-ALL cells may be 'addicted' to DSB repair mechanisms to survive spontaneous/induced DSBs and targeting these pathways should sensitize them to the lethal effect of DSBs.

DSBs are usually repaired by two major repair pathways, homologous recombination (HR) repair and non-homologous end-joining (NHEJ) [4]. While NHEJ plays a major role in non-proliferating cells, HR works predominantly on broken replication forks and usually depends on *BRCA1, BRCA2, PALB2, RAD51* paralogs (*RAD51B, RAD51C, RAD51D, XRCC2, XRCC3*), *RAD51*-mediated pathway [5]. However, in *BRCA*-deficient cells displaying low expression of at least one of these genes, *PARP1*-mediated base excision repair (BER) and alternative nonhomologous end-joining (Alt-NHEJ) and/or *RAD52-RAD51*-dependent alternative HR mechanisms protect cells from the lethal effect of DSBs [6]. Therefore, *PARP1* and/or *RAD52* are attractive targets to eliminate *BRCA*-deficient leukemia cells. mRNA microarray gene expression analyses of 117 T-ALLs and 7 normal bone marrow samples revealed wide-range expression levels of HR genes in T-ALLs (Figure 1(A)) suggesting that some leukemias from individual patients were *BRCA*-deficient. Moreover, the analysis of 12 ETP-ALLs and 40 T-ALLs indicated downregulation of *BRCA1, BRCA2,*

CONTACT Tomasz Skorski, tskorski@temple.edu, Department of Microbiology & Immunology, Temple University Lewis Katz School of Medicine, 3400 N. Broad Street, MRB 548, Philadelphia, PA 19140, USA.

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XRCC2, *RAD51B*, *RAD51C*, and *RAD51* in ETP-ALLs when compared to T-ALLs (Figure 1(B)) suggesting that numerous ETP-ALLs were *BRCA*-deficient. Although mutations in HR genes are extremely rare in T-ALL [7], these data implicate a malfunctioning of *BRCA-RAD51* repair pathway (BRCAness) in a cohort of T-ALLs, especially in ETP-ALLs due to lower levels of at least one protein in *BRCA*-mediated HR. At the same time, expression of *RAD52* seemed upregulated in ETP-ALLs, and *PARP1* and *LIG3* were unchanged (Figure 1(B)). The mechanisms responsible for relatively high frequency ETP-ALLs displaying "BRCAness' are currently not known. Genetic aberrations frequently detected in ETP-ALLs, such as *RUNX1* mutations may be responsible for this phenomenon [8,9].

We hypothesized that targeting *PARP1* and *RAD52* will induce synthetic lethality in T-ALLs/ETP-ALLs displaying 'BRCAness' and spare normal cells. Next, we tested the sensitivity of primary leukemia xenograft (PLX) CD3 + cells from three ETP-ALL patients previously identified as *BRCA*-deficient to DNA repair inhibitors. As controls, we used pan-T cells from healthy donors. All three samples of ETP-ALL cells were more sensitive to *PARP1* inhibitor olaparib and *RAD52* inhibitor 6(OH)-DL-dopa [10] when compared to normal pan-T cells (Figure 2(A)). However, T-ALL PLXs displayed differences in sensitivity to *PARP1* and *RAD52* inhibitors; two samples appeared sensitive whereas three seemed resistant when compared to normal pan-T cells (Figure 2(B)). In addition, we tested the combinations of suboptimal concentrations of *RAD52* inhibitors 6(OH)-DL-dopa and F79 aptamer [11], PARP1 inhibitor olaparib and/or cytotoxic drug Ara-C. Ara-C combined with *PARP1* or *RAD52* inhibitors exerted stronger anti-leukemia activity when compared to individual treatments in *PARP1/RAD52* inhibitor-sensitive ETP-ALL cells, but not in the resistant T-ALL cells (Figure 2(C)).

To determine if the differences in sensitivity to *PARP1* and *RAD52* inhibitors were associated with deficiencies in HR activity, sensitive and resistant leukemia cells as well as normal pan-T cells were transfected with the plasmid-containing HR reporter cassette. HR activity was determined by the restoration of a functional GFP gene and detection of GFP + cells. ETP/T-ALL cells which were sensitive to *PARP1* and *RAD52* inhibitors displayed diminished HR activity in comparison to normal pan-T cells and T-ALL cells resistant to the inhibitors (Figure 2(D)). Moreover, *BRCA1* and *RAD51* foci formation was inhibited in leukemia cells which displayed reduced HR activity and increased sensitivity to *PARP1* and *RAD52* inhibitors (Figure 2(E)).

In summary, our results strongly suggest that the majority of ETP-ALLs and a subset of T-ALLs display the 'BRCAness' phenotype, which predisposes them to be sensitive to *PARP1* and *RAD52* inhibitors. While *RAD52* inhibitors await clinical development, *PARP1* inhibitors such as Lynparza (olaparib), Rubraca (rucaparib), and Zeluja (niraparib) have been FDA approved to treat breast and/or ovarian cancers carrying *BRCA1/2* mutations. We postulate that these inhibitors may be used to improve the therapeutic effect of standard cytotoxic drugs especially in patients with ETP-ALLs.

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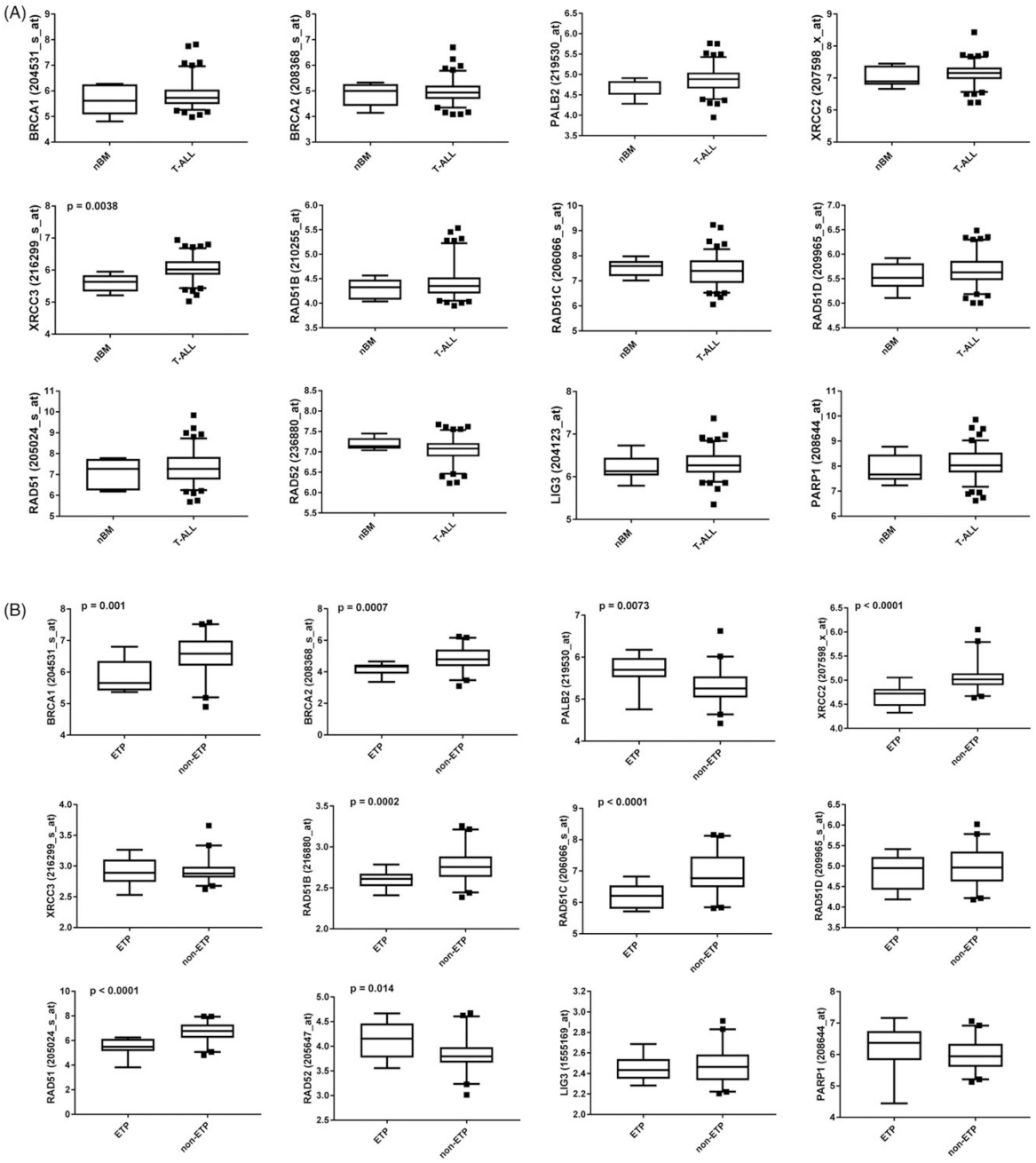

Figure 1.

Expression of HR genes in individual T-ALL/ETP-ALL and normal bone marrow samples. mRNA microarrays genes expression analysis of (A) normal bone marrow samples (nBM, n = 7) and primary T-ALLs (n = 117) from GSE26713 [12] and (B) ETP (n = 12) and non-ETP (n = 40) samples from GSE28703 [7]. Average levels of the probe set signals were obtained from the analysis of Affymetix HG-U133 Plus 2.0 microarrays. Box plots represent the mean ± standard deviation for the selected probes sets (multiple probes sets were tested for each gene). The significance of difference was determined by unpaired two-tailed

Student's *t* test with Welch's correction using GraphPad software. Results with a *p* value <. 05 were considered as statistically significant.

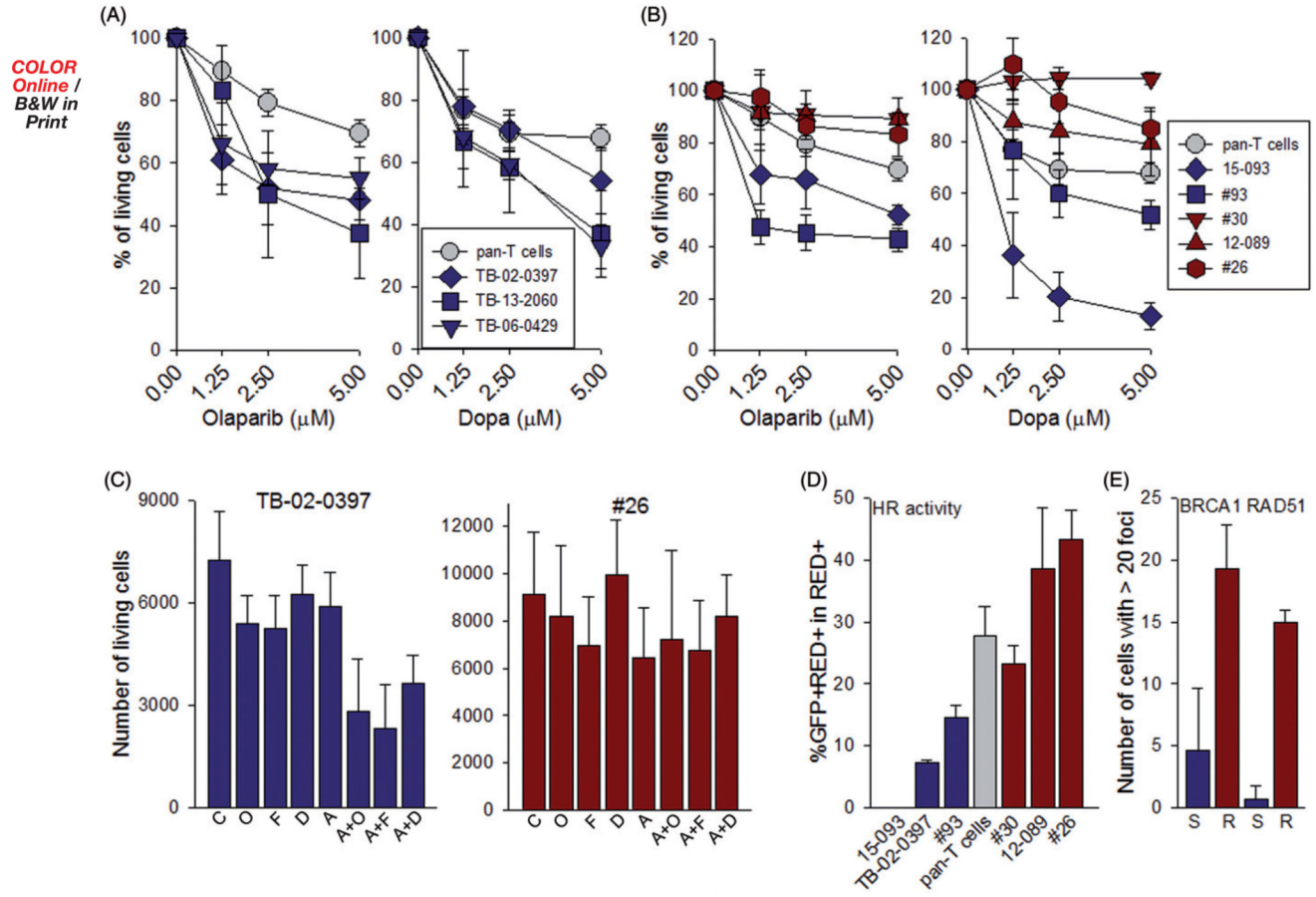

Figure 2.

Sensitivity of ETP/T-ALL cells from individual patients to PARP1 and RAD52 inhibitors and HR activity. (A) ETP-ALL and (B) T-ALL primary leukemia xenograft cells from individual patients were cultured in RPMI 1640 supplemented with 10% FBS, 10% human AB serum, recombinant human stem cell factor (SCF, 50 ng/ml), FLT3 ligand (20 ng/ml), IL-7 (10 ng/ml) and insulin (116 ng/ml) [13]. Normal human pan-T cells were purchased from Stemcell Technologies and cultured in ImmunoCult™-XF T Cell Expansion Medium supplemented with ImmunoCult™ Human CD3/CD28/CD2 T Cell Activator (Stem Cell Technologies). Cells were treated with olaparib or 6(OH)-DL-dopa (Dopa) at 0 and 48 hours. Cell viability was determined at 96 hours by Trypan blue exclusion. Results represent mean ± SD percentage of living cells when compared to untreated cells from triplicates. (C) Cells were untreated (C) or treated with olaparib (O, 1.25 μM), 6(OH)-DL-dopa (D, 1.25 μM), F79 peptide aptamer (F, 5μM), Ara-C (A, 5nM) and a combination of these drugs at indicated concentrations at 0 and 48 hours. Cell viability was determined at 96 hours by Trypan blue exclusion. Results represent mean ± SD percentage of living cells when compared to untreated cells from triplicates; $*p < .025$ in comparison to one drug treatment using Student's *t* test. (D) HR activity in primary cells was examined as described before [14]. Cells ($1–5 \times 10^6$) were co-transfected with 2 μg linearized plasmid carrying HR reporter cassette (HR event restores functional GFP expression) and 0.1 μg dsRedMito vector (for transfection efficiency) using a Human CD34 Cell Nucleofector® Kit (Lonza)

and Amaxa Nucleofector (Walkersville, MD) as described before [14]. After 72 hours the percentage of GFP+/DsRed + cells in DsRed + cells was analyzed by flow cytometry (FACSCanto, BD Biosciences, San Jose, CA) to assess HR activity. Results represent mean ± SD from triplicates/sample; *$p < .02$ in comparison to pan-T cells from healthy donors using Student's *t* test. (**E**) BRCA1 and RAD51 nuclear foci were examined as described before with modifications [15]. Briefly, cells were incubated with 3 μg/ml cisplatin for 12 hours followed by staining with anti-BRCA1 (Calbiochem, OP92 1/500) or anti-RAD51 (Thermo scientific, PA5-27195) antibodies. Secondary antibodies conjugated with Alexa Fluor 488 or Alexa Fluor 594 were applied (Molecular Probes, Eugene, OR). DNA was counterstained with 4′,6′diamedino-2-phenylindole (DAPI). Results represent mean ± SD number of cells with >20 foci/nucleus from three samples per sensitive (S) and resistant (R) groups (50 cells examined per sample); *$p < .02$ using Student's *t* test.