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Anti-oxidant vitamin E prevents accumulation of imatinib-resistant BCR-ABL1 kinase mutations in CML-CP xenografts in NSG mice

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Chronic myeloid leukemia in chronic phase (CML-CP) is initiated by t(9;22), which encodes p210BCR-ABL1 tyrosine kinase that transforms hematopoietic stem cells.[1] CML-CP is leukemia stem cells (LSCs)-derived but leukemia progenitor cell (LPCs)-driven disease. In CML-CP patients ABL1 tyrosine kinase inhibitors (TKIs), such as imatinib, dasatinib and nilotinib, may induce complete cytogenetic or even molecular responses (CCR and CMR, respectively), but it is unlikely that they will cure CML.[2] One of the major reasons of that are BCR-ABL1 tyrosine kinase mutations.[3] These TKI-resistant BCR-ABL1 kinase isoforms usually result from point mutations in the fragment encoding the kinase domain, and numerous strategies have been tested to avoid and overcome the resistance.[4]

Pre-existing and acquired point mutations in BCR-ABL1 kinase are detected in ~23% of imatinib-naive and 50–90% of imatinib-resistant CML-CP patients, respectively.[3] Moreover, BCR-ABL1 kinase mutants resistant to second- and third-generation TKIs emerged, for example due to new and/or compound mutations.[5,6] Thus, CML cells are elusive targets even for the most advanced TKI therapies due to continuous accumulation of point mutations in BCR-ABL1 kinase, which encode the resistance to next generations of TKIs.[7]

Point mutations usually result from misrepair/lack of repair of enhanced oxidative DNA damage arising from reactive oxygen species (ROS).[8] The potential role of ROS-induced oxidative DNA damage in accumulation of point mutations in CML-CP cells was highlighted by our previous studies, showing that not only imatinib-naive but also imatinib-treated LSCs and/or LPCs from CML-CP patients and CML-CP–like BCR-ABL1 transgenic mice contained 2–6 times more ROS and oxidized bases in comparison with their normal

CONFLICT OF INTEREST
The authors declare no conflict of interest.

counterparts.[9,10] In addition, anti-oxidants such as vitamin E reduced ROS and accumulation of TKI-resistant BRC-ABL1 kinase mutations *in vitro*.[11]

To determine whether anti-oxidants may have clinical application, NSG mice were injected with $CD34^+$ cells obtained from five CML-CP patients with no detectable colonies carrying TKI-resistant mutations. These mice were either untreated or treated with vitamin E, which reduced ROS by approximately twofold (Figure 1a).[9,11]

Human $CD45^+$ cells collected from bone marrows and spleens of NSG mice formed colonies *in vitro*, and vitamin E treatment did not affect the engraftment (Figure 1b). Imatinib-resistant clones carrying either E255K or T315I BCR-ABL1 kinase mutations were detected in three out of five untreated xenografts, but in none of the vitamin E-treated samples (Table 1).

In conclusion, we postulate that anti-oxidants such as vitamin E may be applicable in prevention of TKI-resistance, in particular of that driven by BCR-ABL1 kinase mutations. Moreover, as imatinib-treated CML-CP LSCs and LPCs continue to accumulate high levels of ROS resulting in TKI-resistant mutations,[9,10] anti-oxidant treatment could be combined with TKIs to extend/improve the therapeutic effects of ABL1 kinase inhibitors. This speculation is reinforced by the observation that anti-oxidants vitamin E and *N*-acetyl-cysteine reduced the percentage of the resistant clones emerging *in vitro* from imatinib-treated BCR-ABL1–positive cells.[11]

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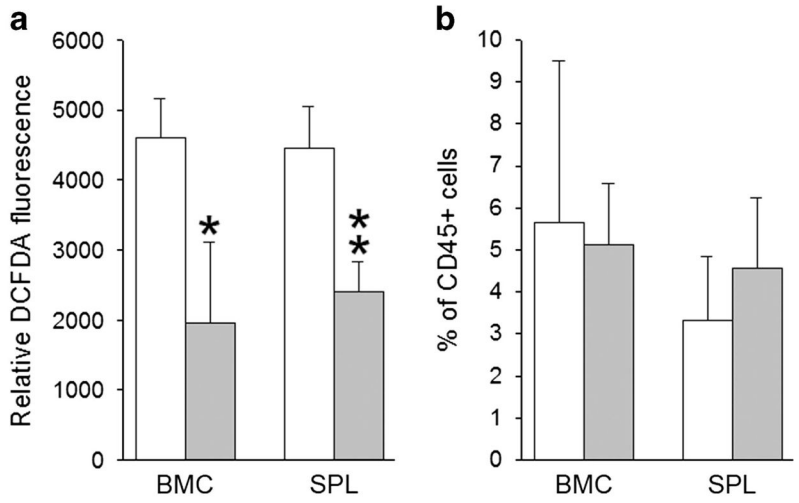

Figure 1.

Vitamin E reduced ROS, but did not affect leukemia engraftment in mice. (**a**) NOD.Cg-*Prkdc*^{scid}/*Il2rg*$^{tm/Wjl}$/SzJ (NSG) mice (Jackson Laboratory) were untreated (white bars) or treated with ~400 international units of vitamin E ((±)-α-Tocopherol (Sigma)) per kilogram of body mass (gray bars) for 1 week. ROS was measured by dichlorofluorescin diacetate (DCFDA) fluorescence in bone marrow cells (BMC) and splenocytes (SPL) as described before;[9] **P* = 0.023, ***P*<0.001. (**b**) NSG mice were treated with 25 mg/kg busulfan (Sigma) 30 h before intravenous injection of 0.5 × 10^6 CD34$^+$ cells from freshly diagnosed CML-CP patients. Vitamin E was administered daily by oral gavage for 12 weeks. Human cells were detected in total BMC SPL populations by flow cytometry using AmCyan-1–conjugated anti-human CD45 antibody (BD Pharmingen).

Table 1

Vitamin E prevents the emergence of imatinib-resistant CML-CP clones carrying BCR-ABL1 kinase mutations

Patient sample	Treatment	Total colonies/ mouse	Imatinib-resistant colonies	Mutation
1	—	2337	7	E255K
	Vitamin E	1116	0	—
2	—	895	5	T315I
	Vitamin E	1539	0	—
3	—	1314	8	T315I
	Vitamin E	1372	0	—
4	—	1126	0	—
	Vitamin E	1218	0	—
5	—	961	0	—
	Vitamin E	1035	0	—

Human CD45 $^{+}$ cells collected from BMC and SPL of the individual leukemic mice described in Figure 1b were incubated with 1 μM imatinib, or left untreated, for 7 days in the absence of growth factors, and then plated in methylcellulose (MethoCult, Stem Cell Technologies) in the presence of 100 ng/ml stem cell factor, 100 ng/ml Flt-3 ligand, 20 ng/ml interleukin 3, 20 ng/ml granulocyte-colony-stimulating factor and 20 ng/ml interleukin 6. Total colonies/mouse and imatinib-resistant colonies were detected and calculated after 7 days. BCR-ABL1 kinase from imatinib-resistant colonies was amplified by reverse transcription-PCR and sequenced as described before.[11] Vitamin E treatment inhibited the appearance of imatinib-resistant colonies; $P = 0.002$, Student's *t*-test.