Hypoxia Activated Prodrug TH-302 Induces Hypoxia-dependent Anti-leukemia Activity in vitro and in vivo

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ABSTRACT

Hypoxia in tumors is generally associated with chemoresistance and radioresistance. Hypoxia-inducible factor-1α (HIF-1α) was expressed in the bone marrow in 58% of primary acute myelogenous (AML) cases by immunohistochemistry (n=156). We have recently demonstrated a marked expansion of hypoxia in leukemia bone marrow (*Benito PLoSOne 2011*) and demonstrated hypoxia promotes chemoresistance in acute leukemias. Normoxic stabilization of HIF-1α using inhibitor of prolyl hydroxylases DMOG, or overexpression through infection with lentiviral non-degradable mutant HIF-1α, significantly diminished apoptosis induced by vincristine and etoposide in REH and Nalm-6 cells. These findings suggest that hypoxia mediates chemoresistance at least in part through HIF-1a. TH-302 is a 2-nitroimidazole of the cytotoxin bromo-isophosphoramide mustard that upon hypoxia-dependent activation induces DNA alkylation, a novel approach to target leukemia. We report the in vitro and in vivo anti leukemia activity of TH-302. In vitro, TH-302 exhibited potent anti-leukemia activity in pre-B ALL (REH, NALM6), AML (OCI-AML3, MOLM-13, KG-1) and CML cell lines (KBM5), with IC50s at 1% O_2 ranging from 0.04μM to 2.3μM. The hypoxic cytotoxicity ratios (aerobic IC_{50}/hypoxic IC_{50}) were 11 and 92 in ALL (REH, NALM6) and 14, 80 and 116 in AML (OCI-AML3, KG-1, KBM5). The drug at 5-7.5μM also exhibited specific hypoxia dependent anti-leukemia activity in primary ALL and AML samples (normoxic vs hypoxic specific apoptosis defined as %AnnV positive cells +/- SEM normalized to untreated control cells) : ALL-1: 5.1+/-0.5% vs 65.3+/-4.2%; ALL-2: 8.3+/-1.9% vs 28.4+/-6.3%; AML 1: 2.6+/-0.2% vs 40.3+/-1.5%). In *in vivo* leukemia models TH-302 (50 mg/kg IP 3 times a week for three weeks) reduced the number of circulating AML cells (control, 13.2+/-5.7 x10^6/ml; TH-302, 2.5+/-2.1 x10^6/ml) and prolonged survival of NSG mice engrafted with primary AML cells compared to the vehicle treated mice (median survival time: TH-302=75 days; Control=56 days; P=0.003). Finally, anti-leukemia efficacy of TH-302 was tested in vitro in combination with chemotherapy commonly used for treatment of AML (AraC, doxorubicin) and with demethylating agents azacitidine (5-AZA) and decitabine (DAC). Combination TH-302 with AraC or with DAC were synergistic in killing OCI-AML3 and MOLM-13 AML cells under hypoxia (combination index values of 0.4 and 0.67 for AraC plus TH-302 in MOLM13 and OCI-AML3, respectively; 0.3 for DAC plus TH-302 in OCI-AML3). In summary, our findings suggest that hypoxia and HIF-1α constitute important factors in the survival of leukemic blasts within the BM microenvironment. The results support targeting hypoxia with a hypoxia-activated drug such as TH-302 to enhance the efficacy of therapeutic regimens in AML. A Phase 1 clinical study of TH-302 in patients with relapsed/refractory hematologic malignancies is ongoing.

BACKGROUND

- We have recently demonstrated a marked expansion of hypoxia in leukemia bone marrow (Benito et al., 2011).
- Hypoxia promotes resistance to radiation and chemotherapy.
- CXCR4 and HIF1a are upregulated in hypoxia (Fiegl et al., 2009) resulting in pro-survival signaling in leukemic cells.
- While HIFs may be valid targets in their own right, bioreductive prodrugs potentially provide a more direct mechanism for sensing and eliminating hypoxic cells.
- Hypoxia-activated prodrugs are a substrate for intracellular one-electron (1e-) reductases, such as cytochrome P450 reductase, which add an electron to the prodrug and therefore convert it to a free radical.
- TH-302 is an inactive prodrug created by the covalent conjugation of 2-nitroimidazole as an oxygen sensor to bromo-isophosphoramide (Br-IPM). In the presence of severe hypoxia and near anoxia, the two imidazole sensor moiety undergoes reduction and the Br-IPM is released in situ. Bromo-IPM is the active alkylating moiety (Figure 1). Good tumor growth inhibition in pre-clinical cancer models has been reported for this prodrug including solid tumors and multiple myeloma (Hu et al., 2010; Sun et al., 2011).

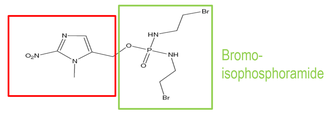

Figure 1. TH-302 structure and mechanism of activation

AIM

The objective of this work was to investigate the role of hypoxia in the leukemic microenvironment and to study anti-leukemia efficacy of the hypoxia-activated prodrug TH-302

References

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RESULTS

HIF-1α is expressed in BM from AML patients

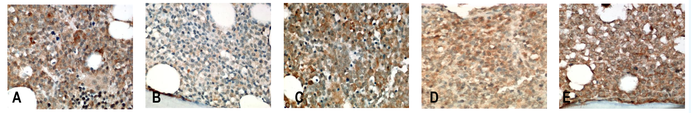

Figure 2. HIF-1α expression was assessed in 156 AML pts with normal karyotype, 89 men and 67 women, with a median age of 66 years (range, 18-90). HIF-1α was expressed in BM of 90 (58%) AML cases, but not in 15 normal BM. Representative images are shown. A) 51 y.o. female, AML M1, diploid, *FLT3/ITD*;B) 46 y.o. female, AML M5, diploid, *FLT3* wild type, C) 36 y.o. female, AML M1, diploid, *FLT3* wild type, D) 78 y.o. male, AML M2, diploid, *FLT3* wild type, E) 58 y.o. female, AML M4, diploid, *FLT3/NPM* wild type.

Normoxic stabilization of HIF-1α significantly diminished apoptosis induced by vincristine and etoposide in ALL cell lines

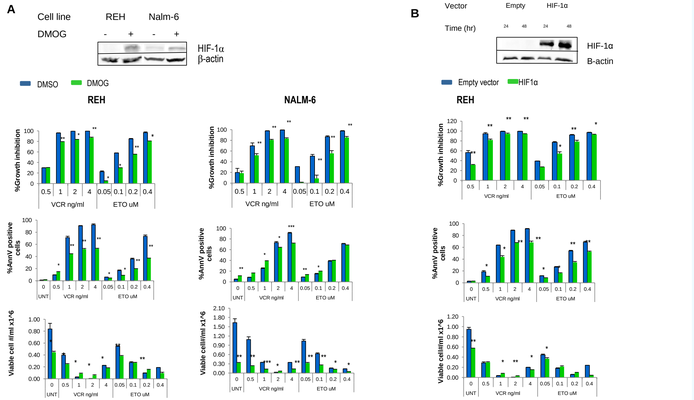

Figure 3. REH cells or NALM6 cells were treated with 100μM DMOG (A) or infected with empty vector or HIF-1α lentivirus and induced with 1μg/ml Doxocycline (REH, B). Western blots show expression of HIF-1α in DMOG (96hr treatment) or Doxocyline treated cells. After inducing HIF-1α expression at 21%O_2, cells were treated with chemotherapy (VCR or ETO). After 72 hrs, effects on cell growth and apoptosis induction were determined by FACS. Growth inhibition was calculated for each group (DMSO, DMOG, Scr or HIF-1α) as the percentage relative to the untreated control. *P<0.05; ** P<0.01.

In vitro TH-302 potent anti-leukemia activity

Cell line	TH-302 incubation (hr)	Total incubation time	Normoxic IC_{50}	Hypoxic IC_{50}	HCR
KBM5	6hr	72hr	16.5	0.14	115.8
KG-1	6hr	48hr	3.3	0.04	80.7
OCI-AML3	6hr	48hr	17	1.25	13.7
Molm-13	6hr	48hr	9.5	0.5	20.5
REH	6hr	48hr	26	2.31	11.3
Nalm6	6hr	48hr	6.9	0.07	92.3

Table 1. Cell lines were exposed to TH-302 for the indicated period of time under normoxic (21% O_2) or hypoxic (1%O_2) conditions and then washed and incubated as indicated under normoxia. Effects on cell growth and apoptosis induction were determined by FACS. HCR: hypoxic cytotoxicity ratio. *P<0.05; ** P<0.01; *** P<0.001. IC_{50} values are uM.

RESULTS

Hypoxia-Selective Cytotoxicity of TH-302: cell lines and primary samples

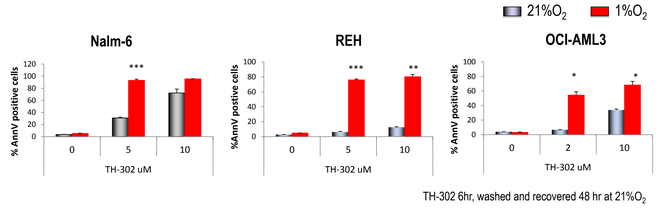

Figure 4. Cell lines or primary ALL or AML samples were exposed to TH-302 for the indicated period of time under normoxic (21% O_2) or hypoxic (1%O_2) conditions and then washed and incubated as indicated under normoxia. Effects on cell number and apoptosis induction were determined by FACS. *P<0.05; ** P<0.01; *** P<0.001. In the AML sample, AnnV is shown for the bulk as well as the CD34+CD38- populations.

TH-302 in vivo anti-leukemia activity

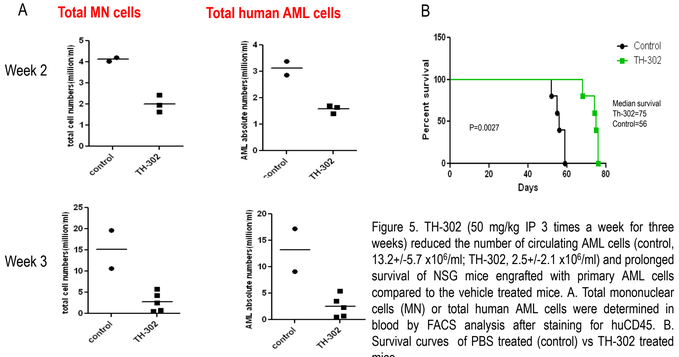

Figure 5. TH-302 (50 mg/kg IP 3 times a week for three weeks) reduced the number of circulating AML cells (control, 13.2+/-5.7 x10^6/ml; TH-302, 2.5+/-2.1 x10^6/ml) and prolonged survival of NSG mice engrafted with primary AML cells compared to the vehicle treated mice. A. Total mononuclear cells (MN) or total human AML cells were determined in blood by FACS analysis after staining for huCD45. B. Survival curves of PBS treated (control) vs TH-302 treated mice.

CONCLUSIONS

- Hypoxia mediates chemoresistance at least in part through HIF-1α
- Hypoxia and HIF-1α constitute an important factor in the survival of leukemic blasts within the BM microenvironment
- Targeting hypoxia with drugs such as TH-302 should enhance the efficacy of the therapeutic regimens in AML. A Phase 1 clinical study of TH-302 in patients with relapsed/refractory hematologic malignancies is ongoing.

RESULTS

In vitro TH-302 anti-leukemia activity in combination with chemotherapy

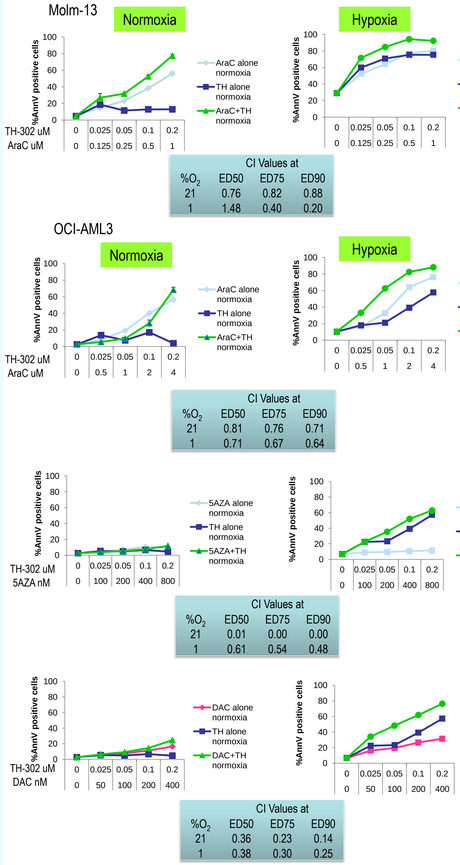

Figure 6. Cell lines were exposed to TH-302 alone or in combination with indicated drugs 72hr under normoxic (21% O_2) or hypoxic (1%O_2) conditions. Effects on cell growth and apoptosis induction were determined by FACS. AraC: Molm-13 and OCI-AML3 ; 5AZA (azacitidine) and DAC (decitabine): OCI-AML3.