ABT-199 and ABT-737 complement the multi-kinase inhibitor TG02 to induce apoptosis in AML cells

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Background

- TG02 is a novel multi-kinase inhibitor currently in Phase 1 trials for AML and CML, with a unique spectrum of molecular targets [Reference 1].
- We have previously shown that TG02 significantly downregulates MCL1 and XIAP but not BCL2 in AML cells at nanomolar concentrations [Reference 2].
- ABT-737 and ABT-199 are Bcl-2 mimetics which inhibit BCL2, but their effects are impaired in cells which over-express MCL1.
- All three agents are capable of targeting dormant as well as proliferating leukaemia cells and thus have the potential to reduce relapse risk, but cellular responses are heterogeneous.

Aims

- To determine whether basal expression levels of BCL2 or MCL1 in patient samples correspond to sensitivity to TG02.
- To determine whether a BCL2 inhibitor increases sensitivity to TG02.
- To determine whether the decrease in cell number induced by the combination of agents is a result of apoptosis.

Methods

- Cells
  KG1a, OCI-AML3 and MV4.11 cell lines and presentation or relapse bone marrow or peripheral blood samples from untreated patients with AML were used in this project.

- Cell viability measurements (patient cells)
  - Primary cells used to generate results in Figure 2 were cultured in triplicate at 1 x 10^6/ml using fibronectin-coated wells in serum-free medium supplemented with 20 ng/ml IL-3, 20 ng/ml IL-6, 50 ng/ml SCF, 100 ng/ml SDF-1 and 50 ng/ml TPO. Cells used to generate results in Figure 6 were cultured in RPMI with 10% FCS supplemented with 20 ng/ml each of IL-3, IL-6, SCF and 25 ng/ml G-CSF.

- Cell growth inhibition/cell death measurements in cell lines
  - KG1a, OCI-AML3 and MEX113 cells were cultured for 24 hours in RPMI with TG02 (Tragara Pharmaceuticals), ABT-737 (Seppro) and ABT-199 (Active Biochemicals). The decrease in the number of viable cells was measured cytometrically using alamar blue.

- Determination of synergy was by the Chou and Talalay method, using CalcuSyn software.

- BCL2 and MCL1 measurements
  - BCL2 and MCL1 were measured in CD2-depleted presentation samples from AML patients by quantitative real time PCR. Details have been published previously [Reference 2].

- Apoptosis measurements
  - Epithelial of bone and MCL that were only exposed upon activation were measured using clone TC100/161 for bak (Millipore) and clone 3 (Transduction Labs) for Bax. Active, bax, bak, active caspase 3 and Annexin V were measured 16 hours post-treatment.

Results

**Figure 2. BCL2 over-expression is a resistance factor to TG02**

48 hour sensitivity to 100 nM TG02 was measured in a cohort of 22 samples. The in vitro toxicity of TG02 was inversely related to basal expression levels of BCL2 (P=0.001), but not MCL1.

**Figure 3. Sensitivity of KG1a, MV4.11 and OCI-AML3 cells to TG02, ABT-199 and ABT-737**

**Figure 4. TG02 synergises with ABT-199 and ABT-737 to induce loss of viable cells**

**Figure 5. BAX and BAX are activated by the combination of TG02 with either ABT-199 or ABT-737 in KG1a cells**

**Figure 6. Sensitivity of primary AML samples to TG02, ABT-199 and ABT-737**

**Figure 7. Timecourse for BAX activation in response to TG02 and ABT-199**

The graphs also indicate the percentage loss with each compound at 18 hours. TG02 - dark markings, ABT-199 - light markings. (i) mean +/- SD for five samples; (ii) data from a sample (AML12) that was more sensitive to ABT-199 and (iii) another (AML18) that was more sensitive to TG02.

**Disclosure statement**
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Summary and conclusions

We have shown an association between BCL2 over-expression and resistance to TG02 in primary AML samples. In KG1a cells we show synergy between TG02 and ABT-199 or ABT-737 at 24 hours, and find induction of apoptosis is considerably higher with the combinations than with the individual agents.

AML samples have a heterogeneous response to all three agents. TG02 was more toxic than the ABT-199 and ABT-737 at equimolar concentrations, but the summarised data indicate that the combinations are at least as effective as TG02 alone.

We conclude that the cytotoxic actions of TG02 and ABT-737 or ABT-199 are complementary.

**References**


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