Abstract
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Comparison of the PI3K- Inhibitors TGR-1202 and GS-1101 in Inducing Cytotoxicity and Inhibiting Phosphorylation of Akt in CLL Cells *in vitro*

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Introduction

1. Chronic lymphocytic leukemia (CLL) is classically defined as an indolent B-cell malignancy. However, patients may require repeated therapies for progressive disease.

2. Despite numerous available therapies, clinical responses may be incomplete and/or of relatively short duration. Therapy is commonly complicated by cytopenias and infections.

3. The PI3K/Akt pathway is known to be a central mechanism by which CLL lymphocytes survive and evade apoptosis. It is known that constitutive activation of Akt promotes CLL lymphocyte survival, while inhibition of the Akt pathway induces CLL lymphocyte death.

4. There are four isoforms of PI3K, and expression of the δ isoform of PI3K is largely restricted to lymphocytes. Clinical evaluation of PI3K-δ inhibitors, such as GS-1101, has been promising, with responses seen in relapsed and/or refractory CLL patients.

5. TGR-1202 is a novel PI3K-δ specific inhibitor previously demonstrated to inhibit Akt phosphorylation and induce apoptosis in B-cell lymphoma cell lines.

Hypotheses

TGR-1202, a novel PI3K-δ specific inhibitor, induces cytotoxicity and apoptosis, and inhibits Akt phosphorylation in primary CLL lymphocytes.

Methods

CLL lymphocytes were obtained from 7 patients at the Duke University and the Durham VA Medical Centers who were enrolled in IRB approved research protocols. Patients' charts were reviewed to collect and/or calculate prognostic information.

Isolation and purification of CLL cells and determination of molecular prognostic markers were performed in the laboratory.

Cytotoxicity was measured after a 3-day incubation of primary CLL cells with serial dilutions of TGR-1202 or GS-1101 in Hybridoma SFM media using the MTS assay. The fractional cytotoxicity was calculated.

Apoptosis was measured after a 2-day incubation of primary CLL cells with serial dilutions of TGR-1202 or GS-1101 in Hybridoma SFM media using flow cytometric measurement of activated caspase-3 and 7AAD staining.

Phospho-flow cytometry was performed to determine the intracellular content of phosphorylated Akt (S473) in purified CLL lymphocytes. CLL cells were evaluated at a 0 hour time point and after 1 hour of incubation in SFM media with or without anti-IgM or IgD and either TGR-1202 or GS-1101 at serial dilutions. Cells were stained with antibodies to CD5, CD20, CD19, and pAkt. Akt phosphorylation was quantified by median fluorescent intensity (MFI).

Conflicts of Interest

Friedman: Research funding from Rhizen Pharmaceuticals
Miskin: Employment and equity ownership in TG Therapeutics
Viswanadha: Employment in Incozen Therapeutics
Vakkalanka: Employment and equity ownership in Rhizen Pharmaceuticals
Other authors: No financial relationships to disclose

Results

Patient	%IgM	IgM	IgD	IgG	ZAP70	CD38	FISH[1]	Dbl Time (days)[2]	IGHV Status[3]
069-006	76	pos	pos	neg	pos	neg	13q	6593	U
472-003	28	neg	pos	neg	pos	neg	13q	1070	M
325-010	56	pos	pos	neg	neg	neg	normal	15243	M
322-013	90	pos	pos	neg	pos	neg	13q, 17p	128531	U
485-002	48	pos	pos	neg	pos	neg	13q	2655	M
498-002	13	neg	pos	pos	neg	neg	tri 12	1104	M
292-009	67	pos	pos	neg	neg	neg	13q	204	M

Table 1: Patient Sample Characteristics and Prognostic Markers
[1] FISH: fluorescence in situ hybridization to detect interphase cytogenetic aberrations
[2] Dbl Time: White blood cell count doubling time (in days)
[3] IGHV status: M = mutated IGHV; U = unmutated IGHV

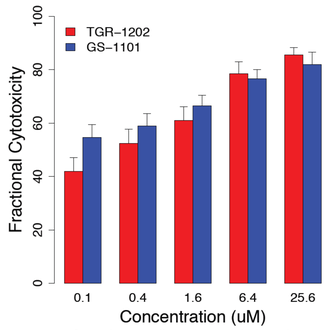

Figure 1: Induction of cytotoxicity in primary CLL cells after 3 days of incubation with TGR-1202 is dose-dependent and is equivalent to GS-1101.

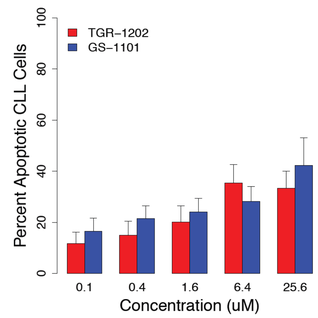

Figure 2: Induction of apoptosis in primary CLL cells after 2 days of incubation with TGR-1202 is dose-dependent and is equivalent to GS-1101.

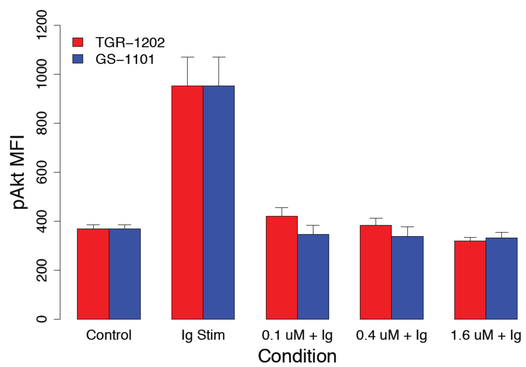

Figure 3: Akt is phosphorylated after cross-linking of the B-cell receptor by anti-immunoglobulin ("Ig Stim"). The addition of TGR-1202 or GS-1101 returns Akt phosphorylation to baseline ("Control") at concentrations between 0.1 to 1.6 µM.

Conclusions

1. TGR-1202 is a PI3K-δ inhibitor that suppresses Akt phosphorylation and induces apoptosis-dependent cytotoxicity in primary CLL cells.

2. The total number of individual patients who provided primary cells is relatively small, yet significant alterations in Akt phosphorylation, apoptosis induction, and cytotoxicity were seen after incubation with TGR-1202.

3. Preliminarily, we observed equal *in vitro* efficacy of TGR-1202 in CLL lymphocytes with high versus low risk prognostic markers.

4. TGR-1202 is equally efficacious to GS-1101 with regards to *in vitro* induction of apoptosis and toxicity, and in suppressing Akt phosphorylation.

5. Our results suggest that TGR-1202 is an effective PI3K-δ inhibitor in CLL *in vitro*, and thus may have benefit as a potential therapy for treating CLL patients.

References

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