Novel PI3Kδ Inhibitor TGR-1202 Demonstrates Cytotoxicity in B- and T-Cell Lymphoma Models and Is Synergistic with A Novel Anti-CD20 mAb, Ublituximab, in Models of Lymphoma

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Rationale to Target the PI3K / AKT / mTOR Pathway

The PI3K / AKT / mTOR pathway plays a key role in multiple cellular processes

Downstream effectors of PI3K/AKT regulate many cellular functions,[1–3] including:

- Metabolism
- Proliferation
- Survival
- Angiogenesis
- Motility
- Differentiation

Components of the PI3K / AKT / mTOR pathway are frequently mutated or altered in common human cancers, leading to constitutive activation of the pathway

- Aberrant activation generally correlates with poor prognosis in some indications[2,4–5]
- Acquired resistance to chemotherapy is associated with aberrant activation[5–6]

Targeting the PI3K / AKT / mTOR pathway is known to arrest tumor growth and induce cell death in cancers that are resistant to currently available therapies[1,7]

1. Liu P, *et al*. *Nat Rev Drug Discov* 2009;8:627–644; 2. Courtney KD, *et al*. *J Clin Oncol* 2010;28:1075–1083; 3. Okkenhaug K and Vanhaesebroeck B. *Nature Rev Immunol* 2003;3:317–330. 4. Saal LH, *et al*. *Proc Natl Acad Sci USA* 2007;104:7564–7569; 5. Huang W-C and Hung M-C. *J Formos Med Assoc* 2009;108:180–194; 6. Kolasa IK, *et al*. *Cancer Biol Ther* 2009;8:21–26; 7. Perego P, *et al. Biochem Pharmacol* 2010;80:1459–1465.

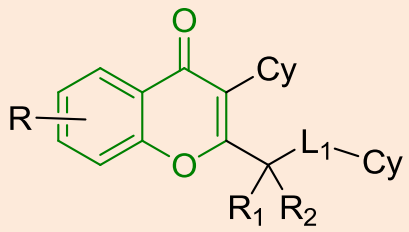
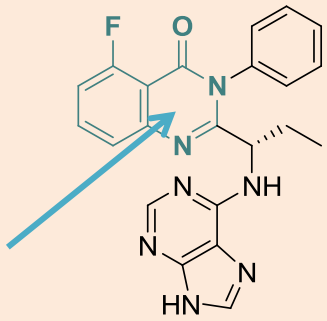
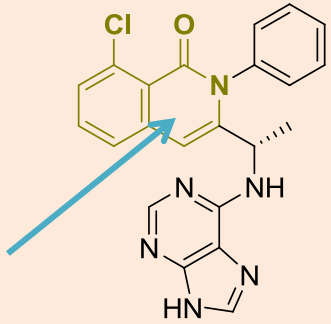
Rationale to Combine PI3K Inhibitors and Anti-CD20 Monoclonal Antibody Therapy

- Rituximab been shown to inhibit the constitutively activated PI3K-Akt pathway in B-NHL cell lines[1].
- Activation of PI3K/AKT is a poor prognostic factor for diffuse large B-cell lymphoma treated with CHOP; however, R-CHOP was able to overcome the poor prognosis associated with activation of PI3K/AKT[2].
- Combination of rituximab and the PI3Kδ inhibitor Idelalisib/CAL-101 was reported to be a highly active regimen for indolent lymphoma[3].
- ***In spite of this strong rationale for combination, there has been limited to no detailed preclinical data of these combinations in lymphoma.***

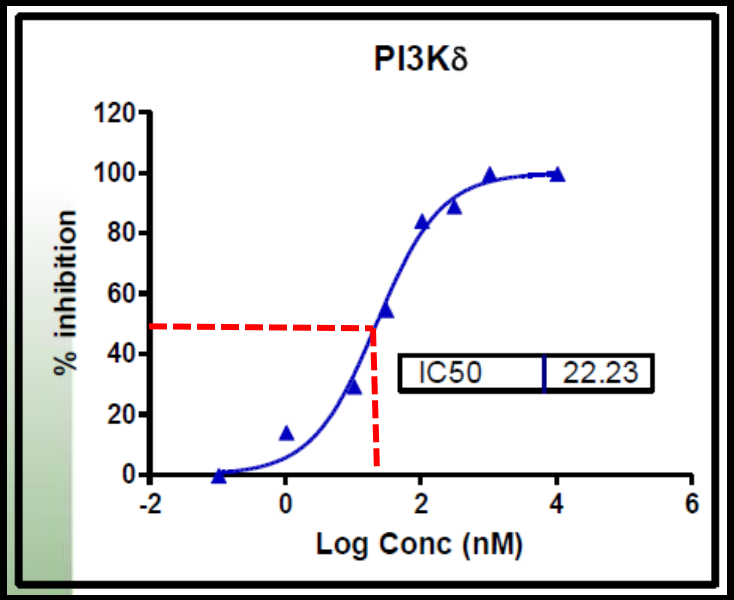
([1] Suzuki et al. Oncogene 2007; [2]Xu et al. Ann Hematology 2013; [3]Leonard et al. ASCO 2013)

Three PI3K Inhibitors in Clinical Development

- Idelalisib is a first-in-class PI3Kδ inhibitor, and has shown promising activity in indolent lymphoma.
- IPI-145 is a PI3Kγ/δ inhibitor that has demonstrated promising activity in both B- and T-cell lymphoma.
- Idelalisib and IPI-145 share high structural similarity and contain nitrogen based heterocyclic backbones that are associated with hepatotoxicity.
- TGR-1202 has a different backbone designed to potentially minimize liver toxicity while preserving delta specificity.

TGR-1202	Idelalisib	IPI-145
		

TGR-1202 Selectively Inhibits PI3Kδ



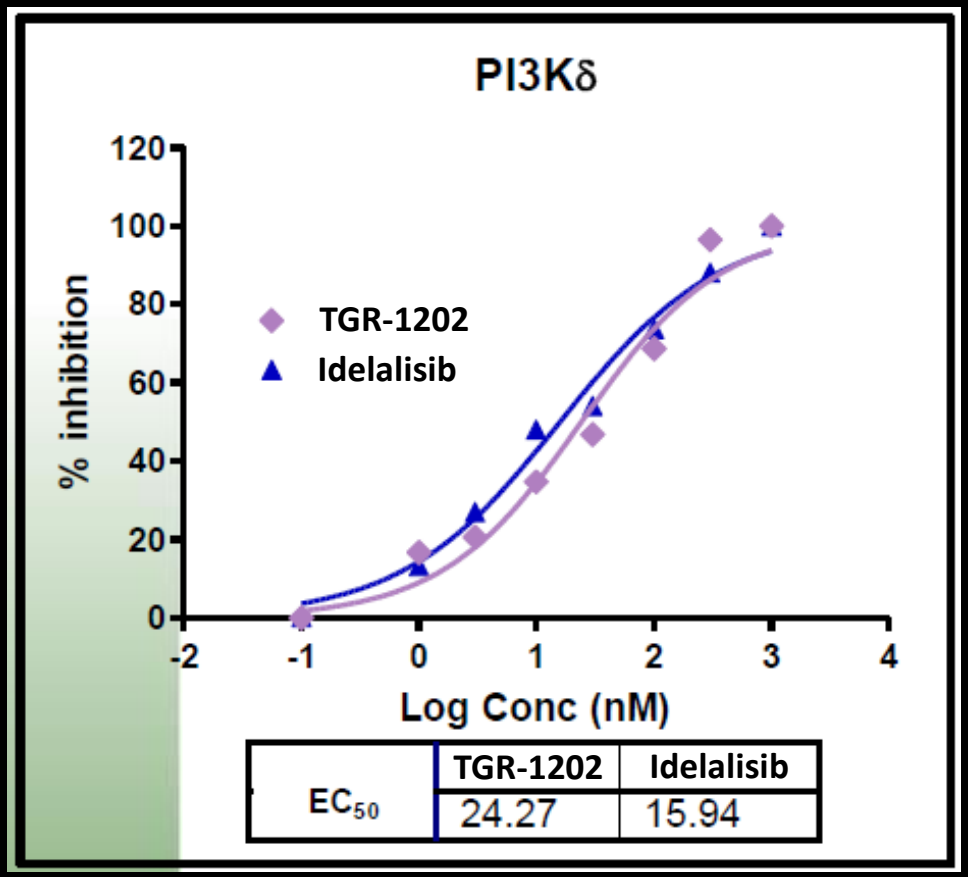
PI3Kδ activity was determined using the recombinant enzyme.

Fold-selectivity of Clinical Stage PI3Kδ Inhibitors				
Isoform	**α**	**β**	**γ**	**δ (IC50)**
TGR-1202	**>10000**	**>50**	**>48**	**1 (22 nM)**
[1]Idelalisib	**>300**	**>200**	**>40**	**1 (2.5 nM)**
[2]IPI-145	**>640**	**>34**	**>11**	**1 (2.5 nM)**

[1]Lanutti et al. ASH 2009, [2] Palombella et al. Keystone Symposium 2013

TGR-1202 Selectively Inhibits PI3Kδ

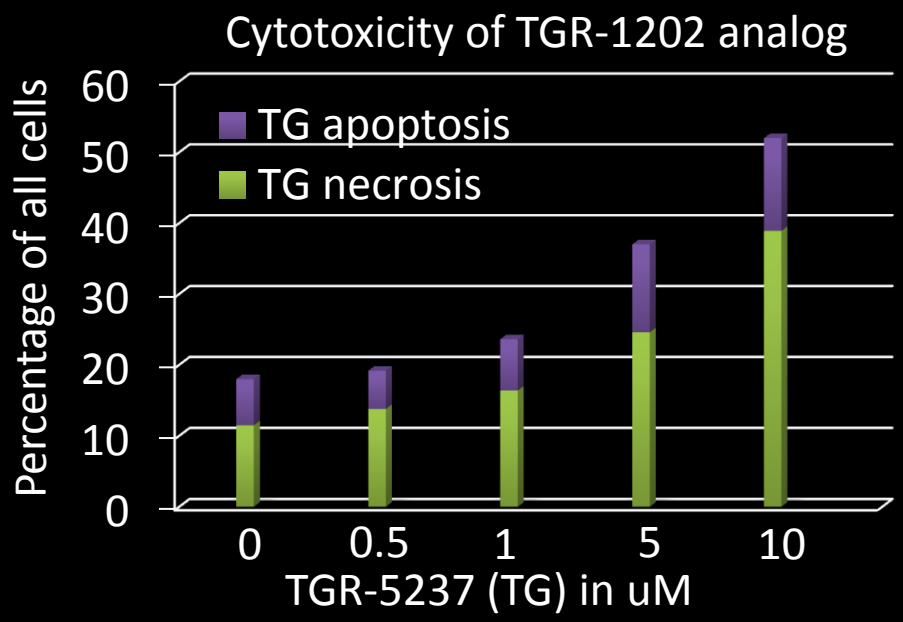
EC50 of TGR-1202 and Idelalisib in a cell based assay

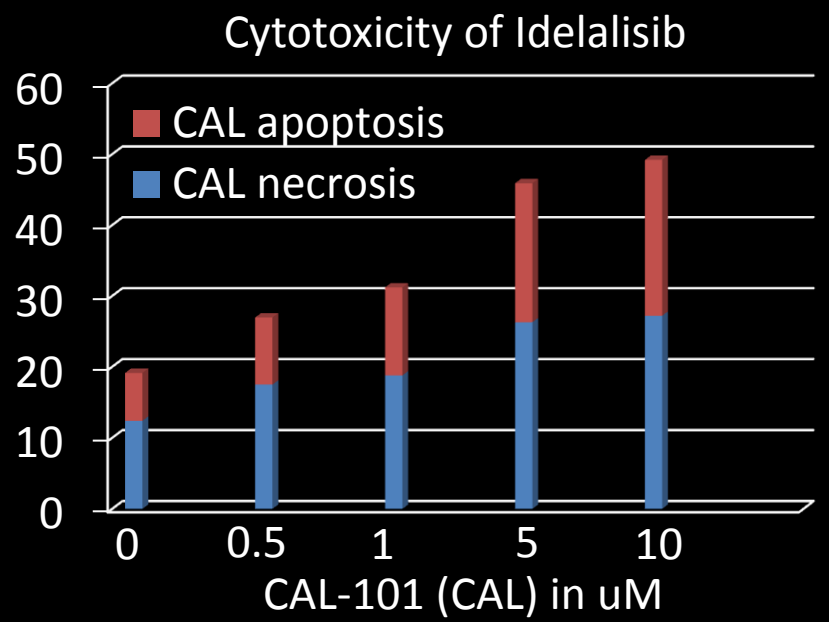


	TGR-1202	Idelalisib
EC_{50}	24.27	15.94

TGR-1202 Induced Apoptosis in B-cell Lymphoma Cell Lines with Comparable Potency to Idelalisib

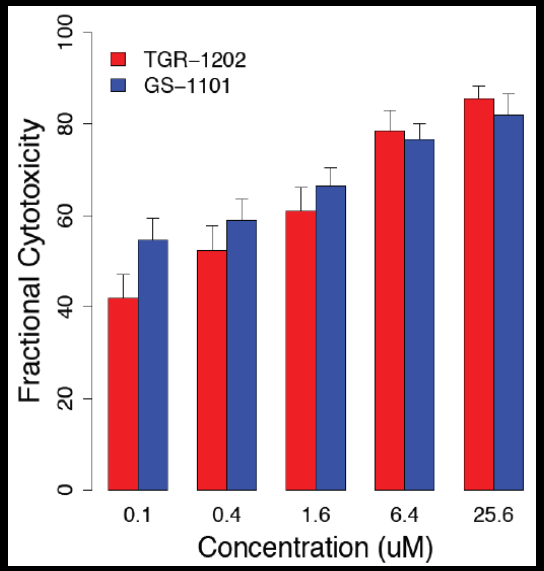
A TGR-1202 analog demonstrated comparable activity of apoptosis in a DLBCL cell line

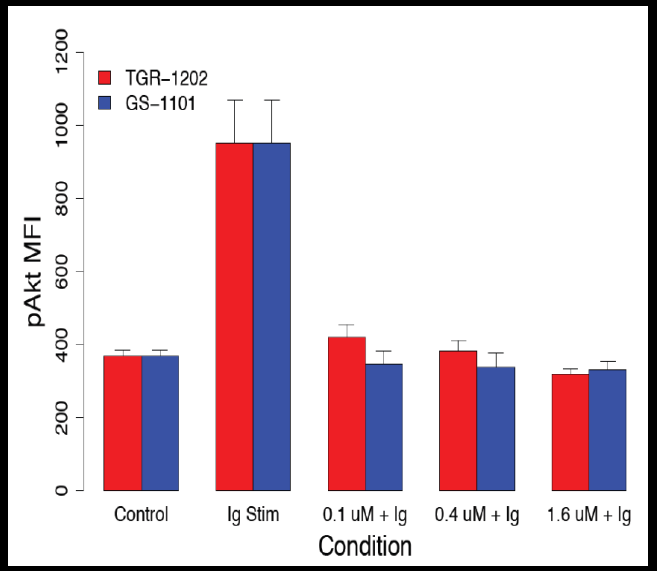


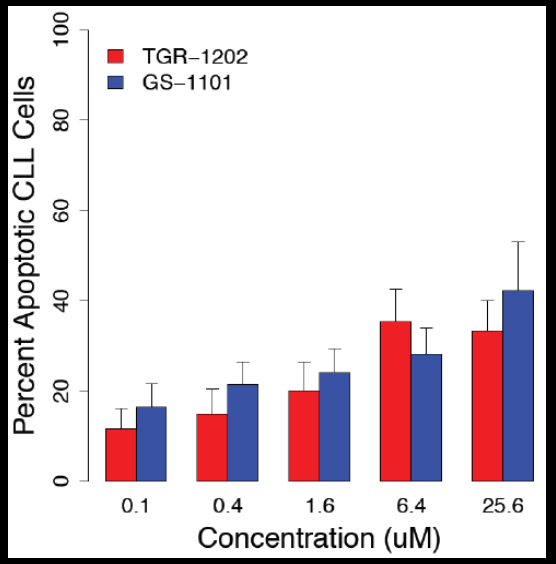


TGR-1202 Demonstrates Comparable Activity to Idelalisib in Patient CLL Cells

In Vitro study comparing TGR-1202 and Idelalisib in CLL patient cells with mixed cytogenetics (n=7)







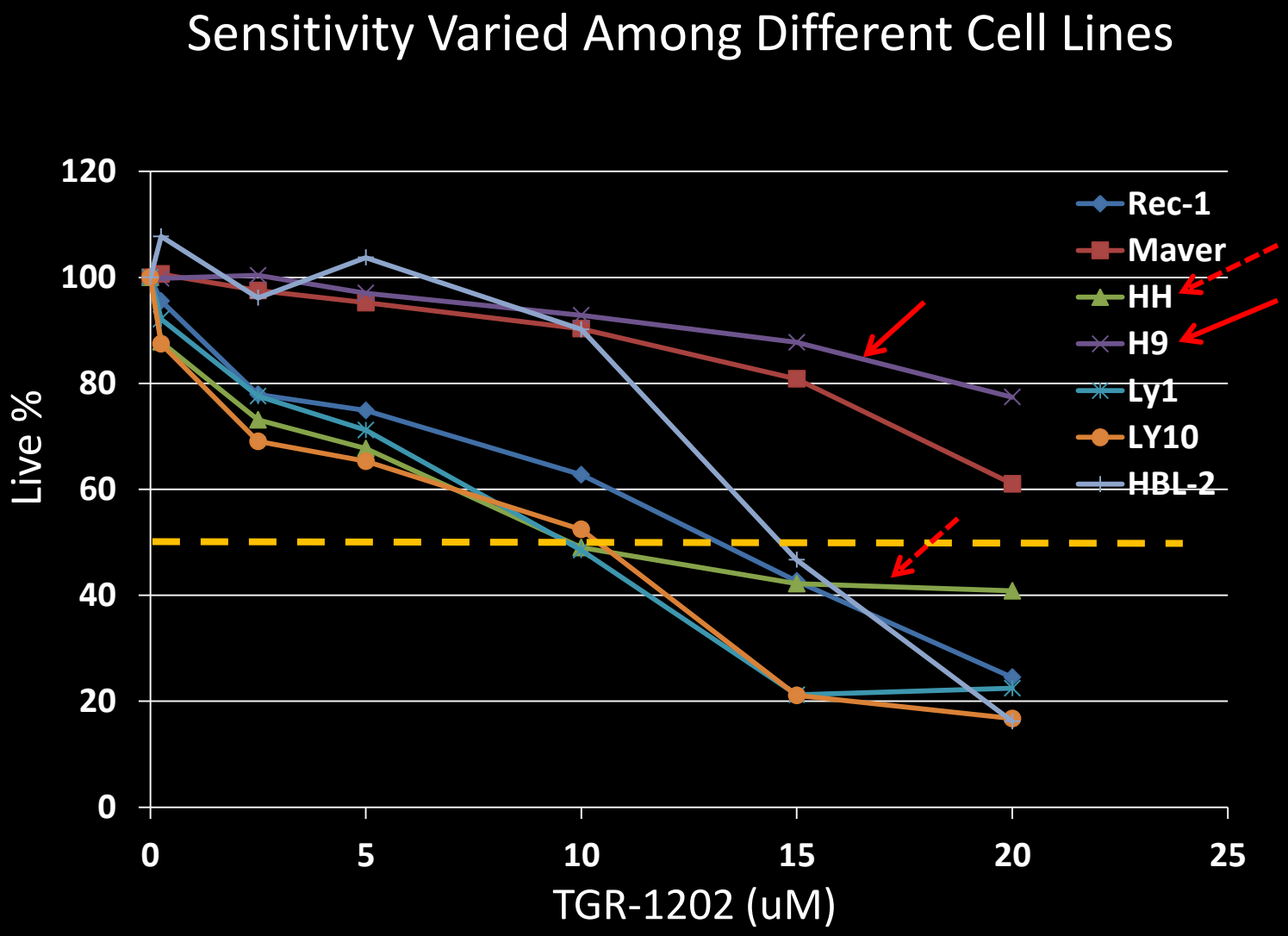
Equivalent dose dependent cytotoxicity

Equivalent suppression of pAKT

Equivalent dose dependent induction of apoptosis

Friedman et al 2012, ASH Poster

Cytotoxicity of TGR-1202 in B- and T-Cell Lymphoma



Effects of TGR-1202 on Cell Cycle Progression in **Diffuse Large B-Cell Lymphoma**

PI3Kδ inhibition produces different cell cycle effects in different DLBCL cells

Treatment	G0/G1	S	G2/M	Sub G0
Control	57.0	7.9	33.5	1.3
5,000 nM	70.5	6.2	19.4	3.2
1000 nM	42.8	12.3	40.9	2.5
200 nM	47.2	8.5	37.5	2.5

LY1 - GCB

Treatment	G0/G1	S	G2/M	Sub G0
Control	59.4	10.3	29.8	0.05
5,000 nM	83.8	7.7	6.4	3.11
1000 nM	76.4	6.0	17.0	0.4
200 nM	37.6	24.2	37.7	0.3

Toledo - DLBCL

Treatment	G0/G1	S	G2/M	Sub G0
Control	58.5	3.2	29.6	8.8
5,000 nM	27.4	0.9	5.7	63.4
1000 nM	45.3	0.7	5.7	50.6
200 nM	53.7	2.6	34.4	10.3

LY10 - ABC

Sub G0 = Apoptosis

Effects of TGR-1202 on Cell Cycle Progression in **Mantle Cell Lymphoma**

PI3Kδ Inhibition Produces different Cell Cycle Effects in MCL Cells

Treatment	G0/G1	S	G2/M	Sub G0
Control	62.6	5.0	24.2	7.7
5,000 nM	46.6	5.9	41.6	2.9
1000 nM	51.9	5.6	39.3	2.7
200 nM	61.6	7.5	27.3	4.7

Jeko

Treatment	G0/G1	S	G2/M	Sub G0
Control	63.8	5.82	27.1	3.9
5,000 nM	33.7	11.8	54.8	0.9
1000 nM	41.0	13.5	46.2	0.7
200 nM	62.9	4.9	26.6	6.4

Rec1

Treatment	G0/G1	S	G2/M	Sub G0
Control	58.7	4.7	35.1	1.2
5,000 nM	36.3	3.1	21.4	39.0
1000 nM	57.6	5.4	31.8	3.8
200 nM	56.4	6.7	35.5	3.6

Maver

Effects of TGR-1202 on Cell Cycle Progression in **Burkitt Lymphoma**

PI3Kδ Inhibition Produces Similar Cell Cycle Effects in BL

Treatment	G0/G1	S	G2/M	Sub G0
Control	63.5	6.3	26.8	1.2
5,000 nM	22.3	19.8	49.5	0.98
1000 nM	37.6	18.6	37.1	0.61
200 nM	44.2	17.2	32.0	0.99

Raji

Treatment	G0/G1	S	G2/M	Sub G0
Control	50.9	11.1	28.5	10.8
10,000 nM	2.5	21.9	65.0	5.0
1000 nM	48.2	7.9	40.1	2.2
100 nM	47.4	11.3	38.0	1.3

Daudi

Understanding the Differential Effects of PI3K Inhibitors on Cell Cycle Kinetics will Provide a Context to Think About Rational Combinations

Sub G0 = Apoptosis

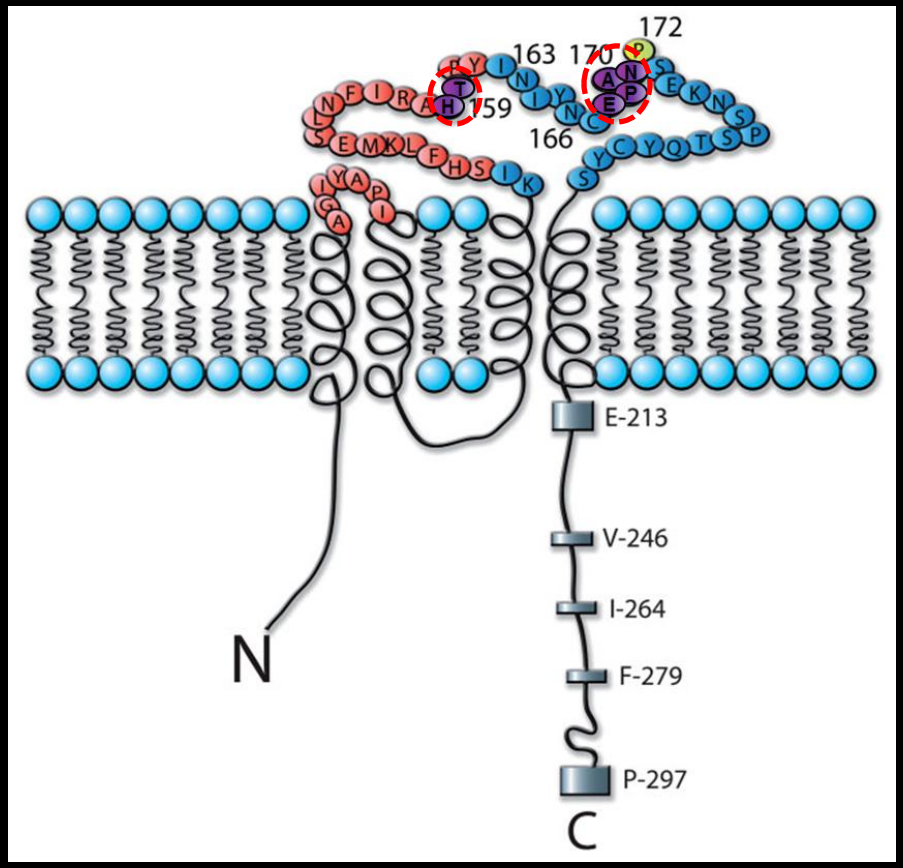
Ublituximab is a Glyco-Engineered Anti-CD20 Monoclonal Antibody

- Ublituximab targets a unique epitope on the CD20 antigen, different from rituximab and ofatumumab.
- A Phase I dose-escalation of ublituximab in patients with rituximab relapsed/refractory NHL reported a response rate of 50% (ASCO 2013).

RED: Amino acids contributing to ofatumumab binding

YELLOW: Amino acids essential for rituximab, but not ofatumumab binding

PURPLE: Core amino acids of ublituximab epitope

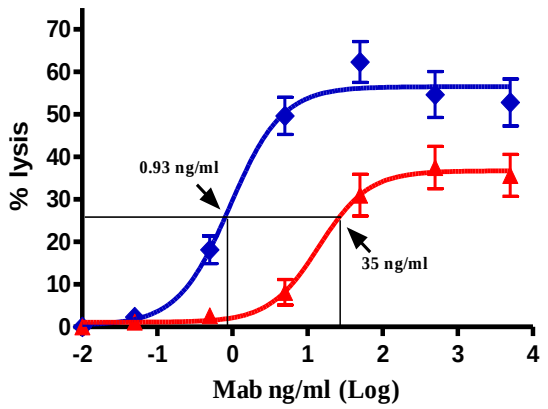


Source: Adapted from Ruuls et al 2008

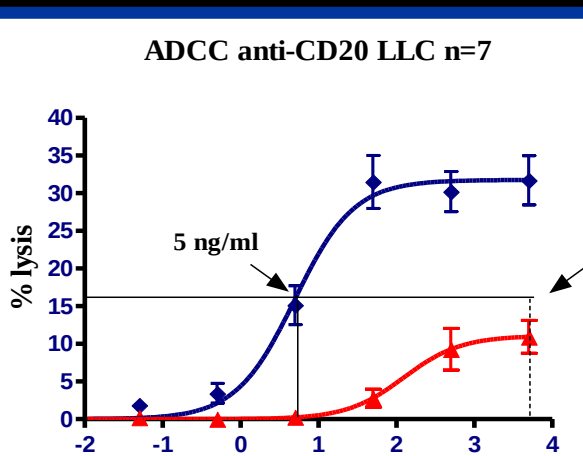
Ublituximab: Superior ADCC Induction Regardless of CD20 Level

Comparison of ADCC mediated by ublituximab and rituximab

Raji
(B-NHL)



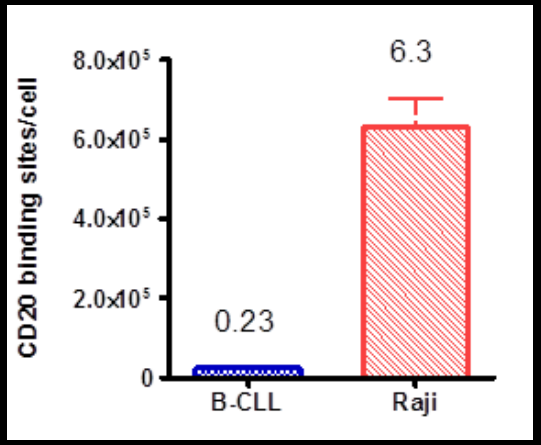
Raji	UBX	RTX
E max % lysis	57	37
EC50 ng/ml	0,93	14,20
50% R603 ng/ml	0,93	35



B-CLL

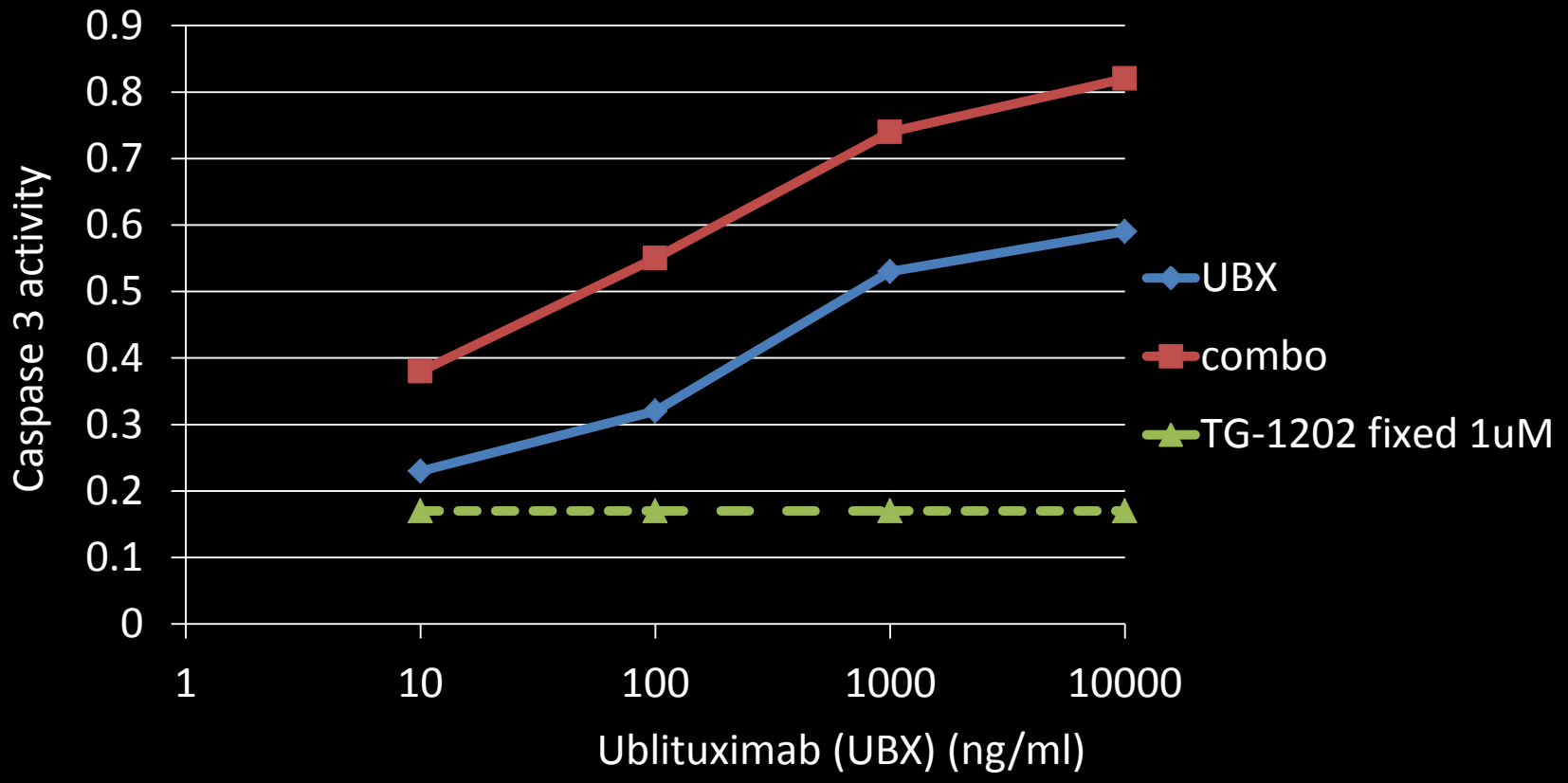
B-CLL	UBX	RTX
Emax % lysis	32	11
EC50 ng/ml	5,00	125,00
50% R603 ng/ml	5	>5000

- Ublituximab exhibits superior ADCC in Raji cells.
- Ublituximab exhibits superior activity even in “low CD20” cases (CLL).



CD20 Expression

TGR-1202 and Ublituximab Synergistically Activated Caspase 3 in the DLBCL Cell Line LY1



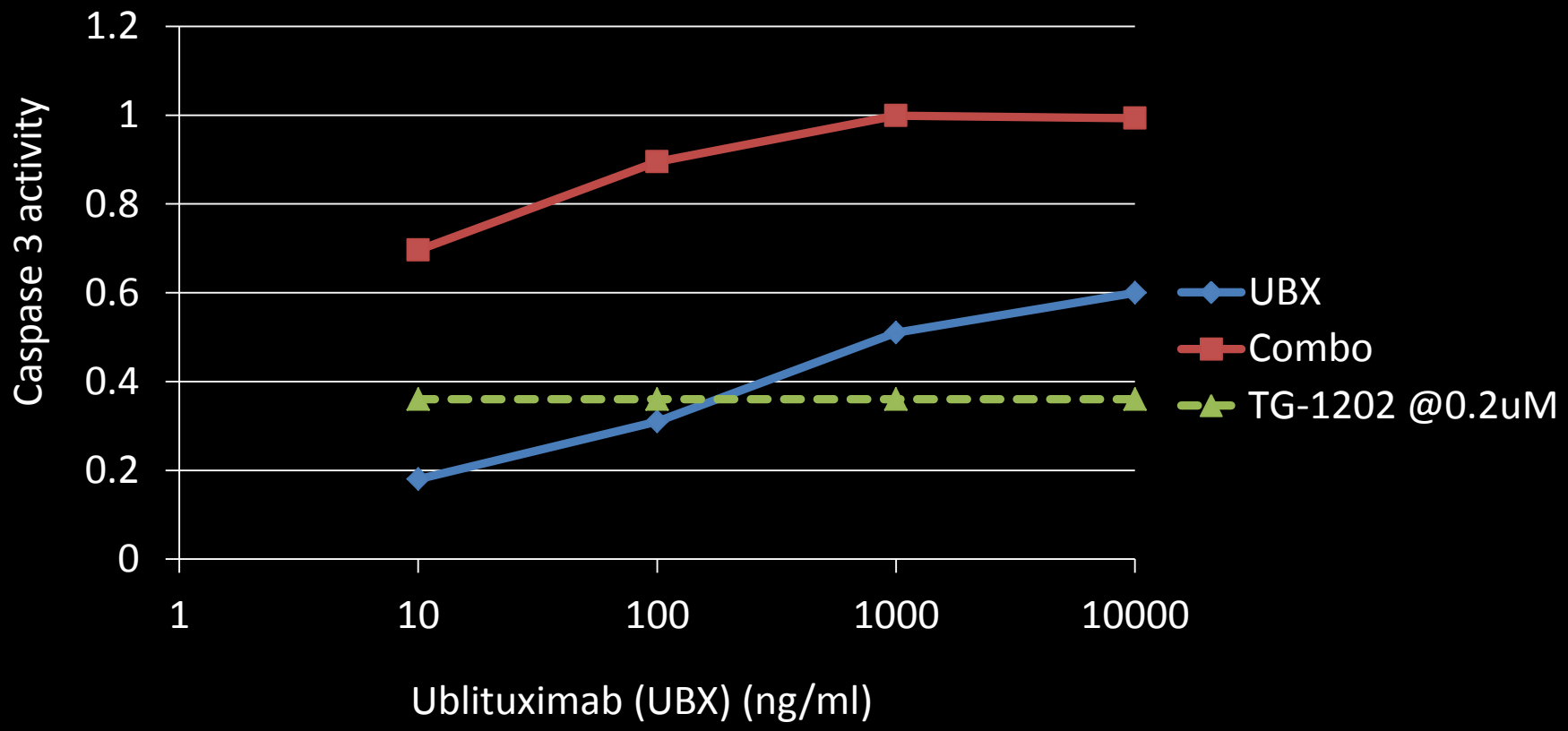
TGR-1202 and Ublituximab Synergistically Activated Caspase 3 in the DLBCL Cell Line LY1

TGR-1202	
Conc nM	Caspase 3
200	0.06
1000	0.17
5000	0.61

UBX	
Conc ng/ml	Caspase 3
10.00	0.23
100.00	0.32
1000.00	0.53
10000.00	0.59

COMBINATION			
TGR-1202 (nM)	UBX (ng/ml)	Caspase 3	C.I.
200	10	0.2715	0.6
200	100	0.3404	1.2
200	1000	0.5902	0.2
200	10000	0.6317	0.8
1000	10	0.3811	0.5
1000	100	0.5523	0.2
1000	1000	0.743	0.1
1000	10000	0.8181	0.1
5000	10	0.7359	0.5
5000	100	0.8339	0.3
5000	1000	0.999	0.0
5000	10000	0.999	0.0

TGR-1202 and Ublituximab Synergistically Activated Caspase 3 in the Burkitt Lymphoma Cell Line Raji



TGR-1202 and Ublituximab Synergistically Activated Caspase 3 in the Burkitt Lymphoma Cell Line Raji

TGR-1202	
Conc (nM)	Caspase 3
200	0.36
1000	0.56
5000	0.80

UBX	
Conc (ng/ml)	Caspase 3
10	0.18
100	0.31
1000	0.51
10000	0.60

COMBINATION			
TGR-1202 (nM)	**UBX (ng/ml)**	**Caspase 3**	**C.I.**
200	**10**	0.696	**0.20**
200	**100**	0.895	**0.04**
200	**1000**	0.999	**0.00**
200	**10000**	0.993	**0.00**
1000	**10**	0.805	**0.49**
1000	**100**	0.939	**0.11**
1000	**1000**	0.928	**0.13**
1000	**10000**	0.866	**0.30**
5000	**10**	0.901	**0.97**
5000	**100**	0.829	**2.03**
5000	**1000**	0.861	**1.52**
5000	**10000**	0.844	**1.80**

TGR-1202 and Ublituximab Synergistically Activate Caspase 3 in Diffuse Large B Cell and Burkitt Lymphoma

TGR-1202 (nM)	UBX (ng/mL)	Caspase 3	C.I.
5000	50	0.999	**0.021**
1000	10	0.421	**0.560**
200	2	0.119	**0.440**

LY10 (DLBCL)

TGR-1202 (nM)	UBX (ng/mL)	Caspase 3	C.I.
5000	50	0.988	**0.336**
1000	10	0.685	**0.548**
200	2	0.136	**0.576**

Toledo (DLBCL)

TGR-1202 (nM)	UBX (ng/mL)	Caspase 3	C.I.
5000	50	0.999	**0.004**
1000	10	0.594	**0.582**
200	2	0.296	**0.393**

Daudi (Burkitt)

TGR-1202 and Ublituximab Synergistically Activate Caspase 3 in Mantle Cell Lymphoma

Synergy Optimized at Higher Concentrations

TGR-1202 (nM)	UBX (ng/mL)	Caspase 3	C.I.
5000	50	0.999	**0.000**
1000	10	0.655	**0.274**
200	2	0.335	**0.506**

Jeko (MCL)

TGR-1202 (nM)	UBX (ng/mL)	Caspase 3	C.I.
5000	50	0.999	**0.000**
1000	10	0.437	**0.603**
200	2	0.213	**0.648**

Maver (MCL)

TGR-1202 (nM)	UBX (ng/mL)	Caspase 3	C.I.
5000	50	0.999	**0.000**
1000	10	0.484	**0.303**
200	2	0.403	**0.132**

Rec-1 (MCL)

Conclusion

- TGR-1202 is a novel PI3Kδ inhibitor with *in vitro* activity in both B- and T-cell lymphoma.
- Ublituximab is a novel anti-CD20 monoclonal antibody with promising clinical activity in patients with relapsed lymphoma.
- TGR-1202 and ublituximab potently synergize in the activation of caspase 3 in B-cell lymphoma models.
- *A multi-center Phase I study with TGR-1202 in combination with ublituximab will be opening in months.*

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