Synergistic Blockade of ABC DLBCL Proliferation with a Selective Inhibitor of IRAK4 in Combination with Inhibition of the B-Cell Receptor Signaling Network

Divya Chaudhary[1*], Nancy Wood[1*], Donna L. Romero[1*], Shaughnessy D. Robinson[2*], Jeremy R Greenwood[2*], Mee Shelley[2*], Michael Morin[1*], Rosana Kapeller[1*] and William F Westlin[1]

[1]Nimbus Discovery, Inc., Cambridge, MA, [2]Schrödinger, Inc., New York,

ABSTRACT #3833

Toll-Like Receptor (TLR) and IL-1 signaling is mediated by the adaptor protein MyD88 through IRAK4 activation. TLR and IL-1 family ligands activate NFκB through this pathway and stimulate proliferation and cell survival, as well as induce cytokine and chemokine production that can amplify tumor cell survival. The gain-of-function L265P mutation in MYD88 occurs in ~30% of patients with activated B-cell like diffuse large B-cell lymphoma (ABC DLBCL) and ~90% of Waldenström's macroglobulinemia. Therefore, inhibition of IRAK4 may be therapeutically relevant in hematologic malignancies containing MyD88 mutations. Recent clinical results with kinase inhibitors strongly support a role for signaling through the B-cell receptor (BCR) pathway in the progression of hematological malignancies including ABC DLBCL. Here we explore the potential therapeutic application of combining a selective IRAK4 inhibitor, ND-2158, with BTK, SYK and PI3Kδ inhibitors.

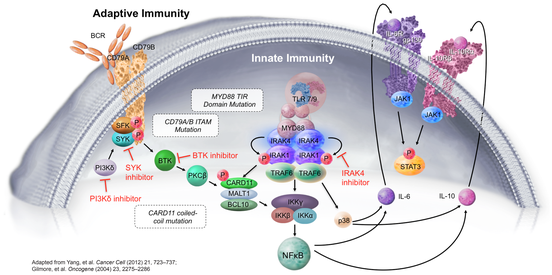
ND-2158, a potent (Ki of 1.2 nM) and highly selective IRAK4 inhibitor has been previously shown to be effective in reducing the proliferation of ABC DLBCL cell lines expressing MYD88 L265P mutant but lacks activity on cell lines that are not dependent on MYD88/NFκB activation (GCB DLBCL).

Here we show:

- Synergistic blockade of ABC DLBCL OCI-LY10 proliferation when ND-2158 is used in combination with inhibitors of either BTK (ibrutinib), PI3Kδ (GS-1101), or SYK (P505-15; BIIB057).
- Combination of ND-2158 with BCR signaling inhibitors enhances the potency, making it comparable to single agent potency blocking cytokine expression in immune cells, that is independent of BCR signaling. The IC_{50} for ND-2158 in blocking proliferation of OCI-LY10 cells shifted from an average value of 6 μM when used as a single agent to 0.19, 0.05, or 0.15 μM, when combined with IC_{50} concentrations of ibrutinib, GS-1101 or P505-15, respectively.
- In contrast to combinations with BCR signaling inhibition, ND-2158 combination with lenalidomide failed to demonstrate synergistic activity due to low single agent activity of lenalidomide.

We conclude that IRAK4 activation, in addition to aberrant BCR signaling, contributes to the proliferative capacity of ABC DLBCL. We propose that combinatorial therapeutic approaches, including inhibition of IRAK4, may provide benefit for patients with ABC DLBCL.

Combination with Other Tumor Signaling Inhibitors: Potential for Improved Response Rates, Durability



Adapted from Yang, et al. Cancer Cell (2012) 21, 723–737; Gilmore, et al. Oncogene (2004) 23, 2275–2286

Potency and Drug-like properties of IRAK4 Inhibitor

		ND-2158
Biochemical IRAK4 Kinase Assay	IRAK4 Ki nM	1.2 ± 0.5
Cytokine Production IC_{50} (nM)	THP1 cells (LPS -> TNFα)	130 ± 50
	PBMC (R848 -> TNFα)	211 ± 158
	Whole Blood (R848 -> TNFα)	316 ± 163
Protein Binding	Human PPB % Bound	82
Human in vitro Stability	Cl_{int} (mL/min/kg) Microsomes	17
Female Lewis Rat PK (SC) 10 mg/kg	$T_{1/2}$ (hr)	1.7
	C_{max} (μM)	2.9
	AUC (μM hr)	6.5
	% F	100
Physical properties	MW(Da), solubility (μM)	425-450, > 300

ND-2158 is Highly Selective Across 334 Kinases

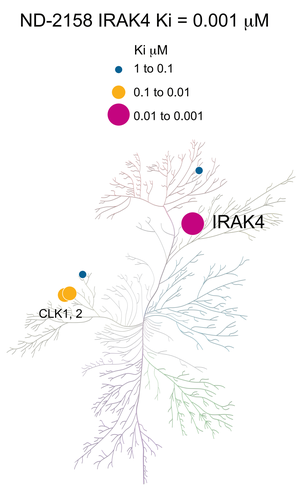


Figure 1: ND-2158 was tested at 10 μM in a 334 kinase panel using radioactive kinase assays at 10 μM ATP (Reaction Biology Corp.). Hits > 60% inhibition were further evaluated in dose response kinase assays for Ki determination. Kinase Ki < 1 μM shown on kinome maps.

In Vivo Proof-of-Mechanism: Potent Inhibition of LPS-Induced Serum Cytokine Production in Lewis Rats

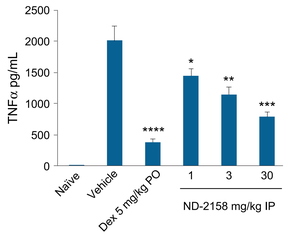


Figure 3: Female Lewis rats (n = 10) were administered ND-2158 IP at 1, 3, or 30 mg/kg, or Dexamethasone PO at 5 mg/kg, or vehicle IP. After 30 minutes, the rats were administered LPS in PBS at 0.1 mg/kg IV. One hour later, rats were bled and rat serum was analyzed for cytokine by ELISA (eBioscience). **** p < 0.0001; *** p < 0.001; ** p < 0.01; * p < 0.05, calculated vs vehicle.

ND-2158 Blocks R848 Induced IκBα Degradation in Monocytes from Whole Blood

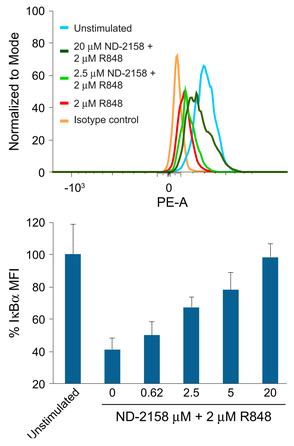


Figure 2: CD14+ gated monocytes in whole blood were stained for IkBα with or without R848-induced stimulation of IkBα degradation for 15 minutes followed by flow cytometry analysis. ND-2158 was pre-incubated with whole blood at the indicated concentrations for 45 minutes prior to stimulation. The left panel shows the representative staining data obtained, with isotype control shown in yellow, and the colors indicating three sample data points as described. In the right panel, data are shown as a % of maximal IkBα mean florescence intensity relative to the average unstimulated condition. Each data point shown is an average of 3 replicates. ND-2158 average IC_{50} was 1.95 ± 0.68 μM (n = 4).

ND-2158 Blocks Proliferation of ABC (OCI-LY10) but not GCB (BJAB) DLBCL Cell Lines

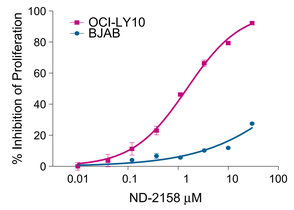


Figure 4: The DLBCL cells lines were plated in duplicate at a density of 50,000 cells per well in 96-well plates along with DMSO as negative control, or different concentrations of ND-2158 as shown. Cell viability at 4 days after drug treatment was determined by MTT assay. A representative experiment for each cell line is shown. Average IC_{50} for ND-2158 in OCI-LY10 cells was 5.2 ± 3.2 μM (n = 15).

Evaluation of Synergism in Cross-Over Combination Study

A

Ibrutinib \ ND-2158 IC_{50}	Dose 1	Dose 2	Dose 3	Dose 4	Dose 5	Dose 6	Dose 7
Dose 1	1:4	1:1	2:1	7:1	20:1	62:1	187:1
Dose 2	1:8	1:3	1:1	3:1	10:1	32:1	95:1
Dose 3	1:16	1:5	1:2	2:1	5:1	16:1	47.5:1
Dose 4	1:33	1:11	1:4	1:1	2:1	7.5:1	22.5:1
Dose 5	1:60	1:20	1:7	1:2	1:1	4:1	12.5:1
Dose 6	1:150	1:50	1:17	1:5.3	1:2	2:1	5:1
Dose 7	1:300	1:100	1:33	1:11	1:4	1:1	2.5:1

B

Ibrutinib \ ND-2158 IC_{50}	Dose 1	Dose 2	Dose 3	Dose 4	Dose 5	Dose 6	Dose 7
Dose 1	1.0	1.0	1.0	0.9	0.9	0.9	0.8
Dose 2	1.0	1.0	1.0	0.9	0.9	0.9	0.8
Dose 3	1.0	1.0	0.9	0.9	0.8	0.8	0.8
Dose 4	0.9	0.9	0.8	0.8	0.7	0.6	0.5
Dose 5	0.9	0.8	0.8	0.6	0.5	0.4	0.3
Dose 6	0.9	0.8	0.7	0.5	0.4	0.3	0.2
Dose 7	0.8	0.7	0.6	0.4	0.3	0.1	0.1

C

Ibrutinib : 2158	187:1	95:1	47.5:1	32:1	16:1	5:1	1:1	1:4	1:7	1:20	1:50	1:300
CI at Fa = 0.8	1.172	0.708	0.515	0.543	0.416	0.299	0.251	0.205	0.325	0.3	0.562	0.727

Figure 5: A cross-over concentration-response grid was set up to measure cell viability effects of two compounds combined together. **A.** Combination indices (CIs) were obtained using the commercial software package CalcuSyn (Biosoft, Cambridge, UK). Drug synergism, addition, and antagonism were defined by CI values of <1.0, 1.0, and >1.0, respectively. For each drug, concentrations above and below the IC_{50} were tested. CI values of < 0.5 are shown in dark green; > 0.5 and < 1 are shown by light green and > 1 are shown in pink. The corresponding concentration combination ratios are indicated. **B.** Fraction affected (Fa) as a combination effect at the indicated concentrations in A, are represented, eg. at Fa = 0.8, CI < 1 demonstrates synergism at the 80% inhibition effect, and the corresponding concentration combination ratios shown in A are synergistic; and at Fa = 0.5, CI < 1 demonstrates synergism at the 50% effect, with the corresponding concentration combination ratios are shown in A. 50% inhibition or Fa = 0.5 (IC_{50}) shifted for one compound in the presence of the second, toward the lower concentration of the compounds, is also depicted. Fa values are color coded as dark green for Fa > 0.9; varying medium shades of green from Fa = 0.8, Fa = 0.7 or 0.6, and Fa = 0.5; light green for Fa < 0.5; and pink for Fa = 0.1. **C.** Synergism is demonstrated at the indicated concentration combination ratios for which CI < 1, at Fa = 0.8 (80% inhibition), as explained in A.

ND-2158 Synergizes with Inhibitors of the B Cell Receptor Signaling Pathway to Block OCI-LY10 Cell Proliferation

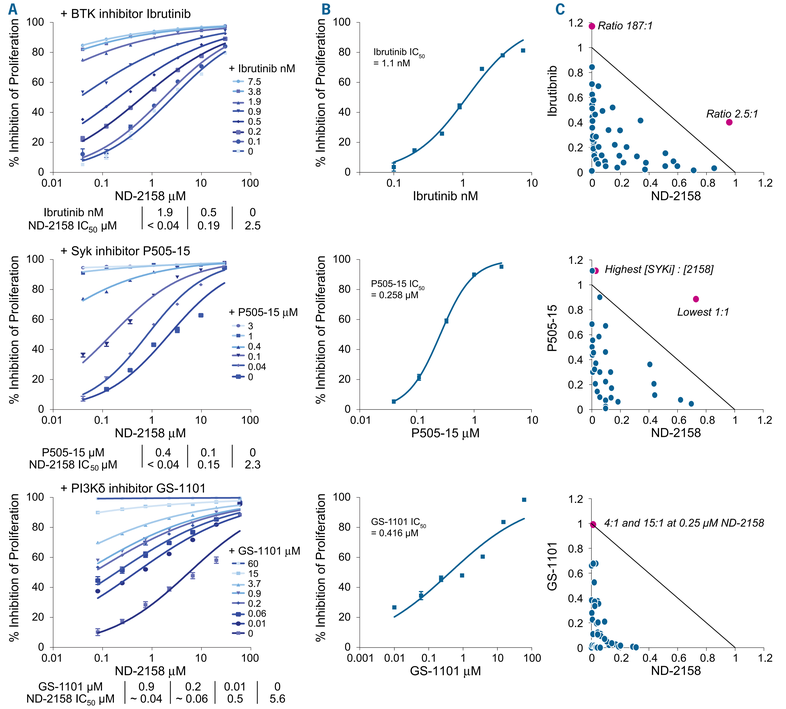


Figure 6: A. ND-2158 potency shifts in the presence of BCR signaling inhibitors, as demonstrated by the left shifted ND-2158 concentration-response curves for OCI-LY10 cell viability in the presence of the respective BCR signaling inhibitors shown. ND-2158 IC_{50} shifts > 60X at the approximate Ibrutinib or P505-15 IC_{50} concentration, and shifts > 140X at the approximate GS-1101 IC_{50} concentration. ND-2158 average IC_{50} as a single agent in these studies was 5.2 ± 3.2 μM (n = 15). **B.** BCR signaling inhibitors concentration-response profiles are shown - Ibrutinib average IC_{50} was 2.5 ± 1.8 nM (n = 10); P505-15 average IC_{50} was 0.48 ± 0.3 μM (n = 12); GS-1101 average IC_{50} was 0.72 ± 0.54 μM (n = 12). **C.** Normalized isobolograms are computed using the calculations shown in Figure 5, and shows synergistic effects of ND-2158 with the respective BCR signaling inhibitors. All CI < 1 are in blue and CI >1 in pink. All experiments were done at least twice and representative results are shown.

Inability to Demonstrate Synergism of ND-2158 Combination with Lenalidomide in OCI-LY10 ABC DLBCL Cells

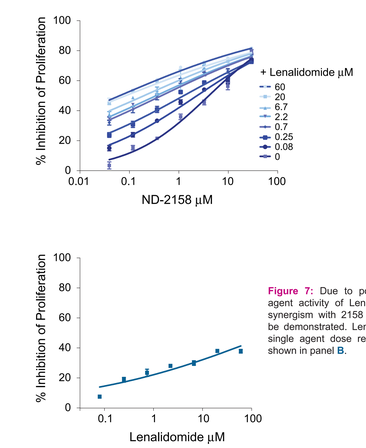


Figure 7: Due to poor single agent activity of Lenalidomide, synergism with 2158 could not be demonstrated. Lenalidomide single agent dose response is shown in panel **B.**

SUMMARY

- ND-2158 is a highly potent and selective IRAK4 inhibitor, that blocks IRAK4-mediate signaling *in vitro* and *in vivo* as measured by inhibition of IκBα degradation and cytokine expression in response to TLR simulation
- ND-2158 is effective in blocking proliferation of ABC (OCI-LY10) but not GCB (BJAB) DLBCL cell lines, suggesting that survival of ABC DLBCL with activating mutations in MYD88 is dependent on IRAK4 signaling
- ND-2158 demonstrates synergistic blockade of ABC DLBCL cell proliferation in combination with BCR signaling inhibitors (BTK inhibitor Ibrutinib, PI3Kd inhibitor GS-1101, and Syk inhibitor P505-15), but shows no significant combination effect with Lenalidomide

CONCLUSIONS

- Inhibition of IRAK4, along with blockade of aberrant BCR signaling, will likely prove efficacious in treating ABC DLBCL
- The results shown here demonstrate drug-sparing combination regimens that may result in increased magnitude and durability of response and overall benefit for patients with ABC DLBCL