Selective and Potent CDK8 Inhibitors Enhance NK Cell Activity and Promote Tumor Surveillance

Boehringer Ingelheim

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INTRODUCTION

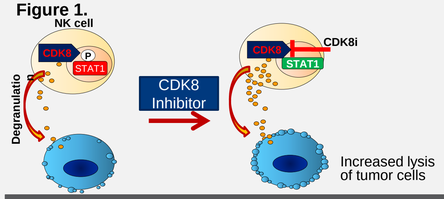
Natural killer (NK) cells play a pivotal role in controlling cancer. Activation of NK cells is tightly regulated by multiple extracellular receptors and internal signaling nodes. The production of perforin and granzyme B, key NK cytolytic molecules, is regulated by the suppressive signaling intermediate Cyclin Dependent Kinase 8 (CDK8) through phosphorylation and inhibition of STAT1^{S727}(1).

We identified highly potent and selective CDK8 inhibitors, including BI-1347 and BI-9811, that we previously reported as valuable probe compound (Hofmann M.H. *et al.*, AACR Annual Meeting 2017, Abstract 4630 (2). CDK8 inhibition promoted activation of NK cells but had no direct cytotoxic activity on the majority of cancer cell lines tested. Single agent treatment with the CDK8 inhibitor BI-1347 increased the survival of mice bearing melanoma and breast cancer allografts.

We evaluated whether increased efficacy could be observed upon activation of both the innate and the adaptive immune system by combining the CDK8 inhibitor with Fc engineered antibodies or SMAC mimetics. SMAC mimetic treatment enhanced T-cell activity and increased NK cell number in the murine EMT6 breast cancer model.

CDK8 Inhibitor: Enhanced NK cell cytotoxicity

Figure 1.



METHODS

Selectivity of our CDK8 inhibitors was analyzed using the Kinase profiling from Invitrogen**. PD biomarker modulation** of STAT1^{Ser727} was analyzed by adding various concentrations of CDK8 inhibitors for 6h to NK92-Mi cells followed by one hour incubation with 100U/ml Interferon beta. **Perforin release** was analyzed by combining NK92-Mi cells with CDK8 inhibitors and analyzing levels of secreted Perforin in the supernatant after 24 h incubation by ELISA (Mabtech AB). **Granzyme B modulation** was analyzed by flow cytometry in murine splenocytes in the presence of 5 ng/ml of IL-15 and 150 nM of BI-1347. **In vivo efficacy** of CDK8 Inhibitors was determined using the murine melanoma B16-F10LUC2 mouse model (Luc2 transfected) or the murine EMT6 mouse model. **NK depletion** with anti-asialoglycoprotein GM1 antibody **CD8+T-cell depletion** with a specific mCD8 antibody. **Depletion of primary leukemia cells from CLL patients** were stimulated with the CDK8 inhibitors and rituximab (10 µg/ml) for 48 h, and the depletion of leukemia cells was analysed by flow cytometry. **ADCC assay** with human NK cells from healthy donors treated with CDK8i over night before addition of radiolabeled HL-60 target cells treated with CD33 mAb (BI 836858) or isotype

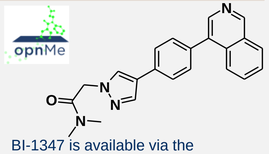
RESULTS

BI-1347 a potent and selective CDK8 inhibitor

Figure 2. A) BI-1347
IC_{50} CDK8: 1 nM ; LE: 0.45; LLE: 5.7
Solubility: 11 µg/ml
Human hep.: 17 %QH
CL mouse QH:14 % ; F mouse: 100%

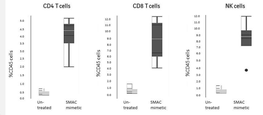
	BI-1347 %Inh. @ 1 µM	BI-1347 IC_{50} [nM]
CDK8	103	1.5
CDK11/CDK19	101	1.7
MLCK	86	530
AURKB	58	810
ICK@IG	51	2,400
FLT3	40	1,400
NTRK1	37	12,000
STK16	31	3,600
GSK3A	1	>20,000
GSK3B	5	>20,000
PIK3CAPIK3R1	11	n.d.
315 additional kinases	<30	n.d.

No effect was observed on all other CDKs tested (e.g. CDK1, CDK2, CDK4, CDK6, CDK7 and CDK9)



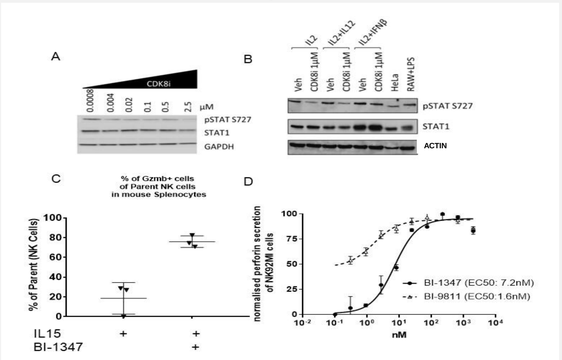
BI-1347 is available via the **opn**ME.com portal from Boehringer Ingelheim

Figure 2. B) Treatment with the SMAC mimetic BI-8382 (3), enhances the number of CD4, CD8 T-cells and of NK cells



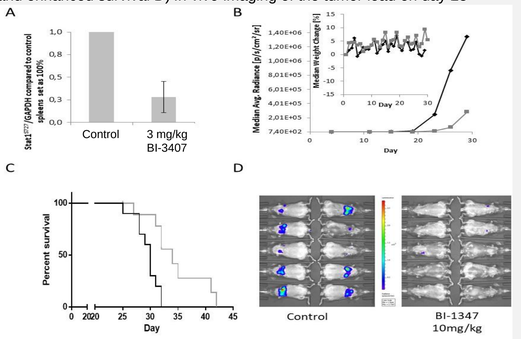
In vitro biomarker modulation

Figure 3. Treatment with BI-1347 resulted in **A)** reduction of pSTAT1^{Ser727} following treatment of NK92-Mi cells **B)** modulation of phospho STAT1^{Ser727} in primary human NK cells from healthy donors by CDK8i (BI-9811, (2)) **C)** increase of Granzyme B positive NK cells in ex-vivo treated splenocytes by BI-1347 **D)** secretion of Perforin in NK92-Mi cells by BI-1347.



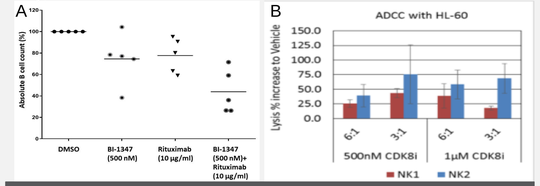
In vivo biomarker modulation and efficacy in monotherapy

Figure 4. A) *In vivo* biomarker modulation in splenocytes analysed 6h post treatment with BI-1347 **B)-C)** Treatment of disseminated B16-F10 mouse model with the CDK8i BI-1347 (qd, p.o.) resulted in reduced tumor growth and enhanced survival D) *In vivo* imaging of the tumor load on day 23



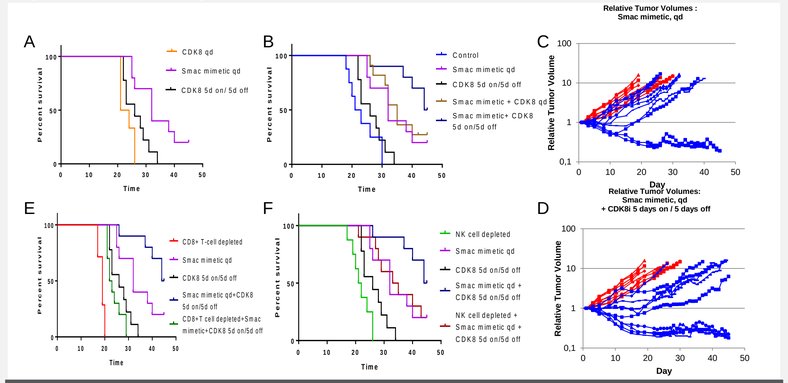
CDK8 inhibitors enhance ADCC of Fc engineered antibodies

Figure 5. A) Depletion of primary leukemia cells from chronic lymphocytic leukemia patients in monotherapy with CDK8 inhibitors, which was increased in combination with Rituximab **B)** Enhanced ADCC by CDK8 inhibitor treated NK cells. Effector NK cells (n=2 healthy donors) were treated with CDK8 inhibitor over night before using for ADCC with radiolabeled HL-60 target cells treated with CD33 mAb (BI 836858) or isotype



Smac mimetic (BI-8382) in combination with intermittent schedule of CDK8 inhibitor (BI-1347) enhances NK cell mediated tumor lysis and survival in the EMT6 murine breast cancer model.

Figure 6. A) Dosing the CDK8 inhibitor (10 mg/kg) or the Smac mimetic (50 mg/kg qd) continuously **B,** The Smac mimetic was combined with CDK8 inhibitor either using a daily dosing of 10 mg/kg of BI-1347 or scheduled dosing, 5 days on / 5 days off (5d on/off) **C-D)** Individual relative growth curves of each individual tumor are shown. **E)** Depletion of CD8+ T-cells in the presence of both the Smac mimetic and CDK8 inhibitor **F,** Depletion of NK cells in the presence of both the Smac mimetic and CDK8 inhibitor



SUMMARY

- We identified potent CDK8 inhibitors that show biomarker modulation (pSTAT1^{Ser727}) which translated into activation of NK cells and in vivo efficacy
- CDK8 inhibitors enhances ADCC of Fc engineered antibodies
- Combination of a Smac mimetic with the CDK8 inhibitor using an intermittent schedule was synergistic and NK cell depletion revealed that the additive effect observed in the combination studies was contributed by natural killer cells
- Pulsatile scheduling of the CDK8 inhibitor BI-1347 permitted activation of NK cells and avoided a hypo-responsive steady state of NK cells.

CONCLUSIONS

We propose new strategies for development and positioning of CDK8 inhibitors in solid tumors by

- using CDK8 inhibitors as mediators of NK cell activity, rather than tumour directed therapy in solid cancers;
- combining CDK8 inhibitors with ADCC enhancing antibody therapeutics or T cell activating anticancer therapies as for example Smac mimetics,
- applying intermittent scheduling in order to avoid NK cell hypo-responsiveness and to minimize safety concerns.

REFERENCES

(1) Putz EM. et al. (2013) Cell Reports 4:437-444

(2) Hofmann MH., Engelhardt H., Carotta S. et al. (2017) Development of Selective and Potent CDK8 Inhibitors that Increase NK Cell Activity, Which Translates in Tumor Surveillance. AACR2017 p Abstract nr. 4630

(3) Reschke M, Impagnatiello MA et al. (2017) BI5: a novel Smac mimetic that triggers tumor cell death and potentiates PD-1 mediated cancer therapy. AACR2017. p Abstract nr. 2330.

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