We directed in vivo inhibitors enhancing the NK cell activity and promote tumor surveillance.

**INTRODUCTION**

Natural killer (NK) cells play a pivotal role in cancer surveillance. Activation of NK cells is tightly regulated by multiple extracellular receptors and internal signaling molecules. The product of CD95- and perforin-like NK cell cytotoxic molecules is regulated by the suppressive signaling intermediate Cytochrome c (CD8) through phosphorylation and inhibition of STAT3 (1).

We identified highly potent and selective CDK8 inhibitors, including BI-1347 and BI-9811, that we recently reported as valuable probe compounds (Hofmann M.H. et al., AACR Annual Meeting 2017. Abstract 4502). CDK8 inhibition promoted activation of NK cells but had no direct cytolytic 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 efficiency could be observed by coupling 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.

**METHODS**

Selectivity of our CDK8 inhibitors was analyzed using the Kinase profiling by flow kinePhenotyping, PD biomarker modulation of STAT1 signaling in human K562 cells and biologically relevant mRNA expression in human K562 cells.

**RESULTS**

In vitro biomarker modulation and efficacy in monotherapy Figure 4. A) in vitro biomarker modulation in splenocytes analyzed 4h post treatment with BI-1347 (B-E) Treatment of disarmed B16F10 mouse models with the BI-1347 (p.o.) resulted in reduced tumor growth and enhanced survival. B) In vivo imaging of the tumor load on day 23.

CDK8 inhibitors enhance ADC 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 ADC by CDK8 inhibitor treated NK cells. Effector NK cells (m2 healthy donors) were treated with CDK8 inhibitor over night before using for ADC with radioislated HL-60 target cells treated with CD33 mAbs (IS 836585) or isotype.

**SUMMARY**

We identified potent CDK8 inhibitors that show biomarker modulation (pSTAT1 (1-9µM)) which translated into activation of NK cells and increased tumor surveillance.

CDK8 inhibitors enhance ADC 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 reduced cell viability. 

Pursuit of testing the CDK8 inhibitor BI-1347 permitted activation of NK cells and avoided a hypo-responsive steady state of NK cells.

**REFERENCES**


**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 ADC enhancing antibody therapies to T cell activating antibodies as e.g., SMAC mimetics, applying intermittent scheduling in order to avoid NK cell hypo-responsiveness and to minimize safety concerns.

**ACKNOWLEDGMENTS**

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**SELECT AND POTENT CDK8 INHIBITORS ENHANCE NK CELL ACTIVITY AND PROMOTE TUMOR SURVEILLANCE**

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**REFERENCES**