CYC065

Autumn 2018

Clinical CDK2/9 inhibitor with development potential in Mcl-1, MYC and cyclin E dependent cancers

CYC065[1] is a highly-selective, orally- and intravenously-available, 2nd generation inhibitor of cyclin dependent kinases (CDK) 2 and 9. Preclinical data suggest that CYC065 may benefit patients with adult and pediatric hematological malignancies such as CLL, AML, ALL, B-cell lymphomas, multiple myeloma and, certain cyclin E-addicted and MYC-amplified solid tumors, such as HER2+ breast cancer, uterine serous carcinoma and neuroblastoma. Translational biology supports development of CYC065 as a stratified medicine for cancers dependent on Mcl-1, MYC and CDK2/cyclin E for proliferation, survival and resistance to treatment. In a Phase 1 clinical study durable target engagement and durable suppression of the Mcl-1 biomarker were observed after a single dose of CYC065.

Mechanism of Action

The CDK enzyme family act as cell cycle regulators, but are now understood to include pivotal functions in regulation of transcription, DNA repair and metastatic spread. The precise selectivity of individual CDK inhibitors for certain preferred CDK/cyclin protein complexes is key to targeting particular tumor types and avoiding undesirable side effects through non-specific antiproliferative activity. Dysregulated CDKs targeted by CYC065 can drive particular subtypes of cancer:

- CDK2, a driver of cell cycle transition and when dysregulated enabler of G1 checkpoint bypass;
- CDK9, an effector of dysregulated transcription of certain genes (incl. cyclins, Mcl-1, MYC) through phosphorylation of RNA polymerase II.

CYC065 is mechanistically similar to seliciclib, Cyclacel's first generation CDK inhibitor, but with significantly improved metabolic stability, efficacy and potency *in vitro* and *in vivo*. CYC065 causes proportionally greater CDK9 inhibition, leading to improved efficacy in hematological malignancies.

Competitive Positioning

As a selective CDK2/9 inhibitor, CYC065 offers an improved therapeutic window and lower myelosuppressive potential than pan-CDK inhibitors based on preclinical and early clinical data. For example CDK1 inhibition counteracts degradation of Mcl-1 protecting cancer cells from anticancer agent activity.

Three recently approved CDK4/6 inhibitors have validated the class and cell cycle inhibition strategies. Palbociclib (Ibrance®, Pfizer), constitutes an important therapeutic advance, causing prolonged cell cycle arrest and senescence in combination with endocrine therapy (ET) in ER+/HER2- breast cancer.

Ribociclib (Kisqali®, Novartis) and abemaciclib (Verzenio®, Lilly) offer broadly similar benefit to palbociclib in patients with breast cancer in combination with aromatase inhibitors (AI). CDK4/6 inhibition has not been shown to modulate Mcl-1.

CDK9 inhibition induces apoptotic tumor cell death through transcriptional downregulation of cancer cell survival pathway proteins, including Mcl-1. CDK2 inhibition enhances cell cycle arrest and may overcome CDK2/cyclin E dependent resistance to CDK4/6 inhibitors.[3]

Cancer cells often overexpress Mcl-1 to escape chemotherapy and/or targeted agents, including inhibitors of other members of the antiapoptotic Bcl-2 family, such as venetoclax (aka ABT-199, Venclexta®, AbbVie). Mcl-1 has therefore emerged as an important target in strategies aiming to suppress expression of pro-survival proteins and regulating cancer cell death. CYC065 targets Mcl-1 expression via inhibition of CDK9. A combination approach to simultaneously suppress Bcl-2 and Mcl-1 has a clear biological rationale.

A Cyclacel-sponsored Phase 1b study is open for enrollment with the goal of evaluating CYC065 in combination with venetoclax in relapsed/refractory CLL patients. Preclinical data demonstrate that the CYC065-venetoclax combination mediated synergistic cell killing in CLL models, irrespectively of 17p deletion, and induced prolonged downregulation of Bcl-2 and Mcl-1.

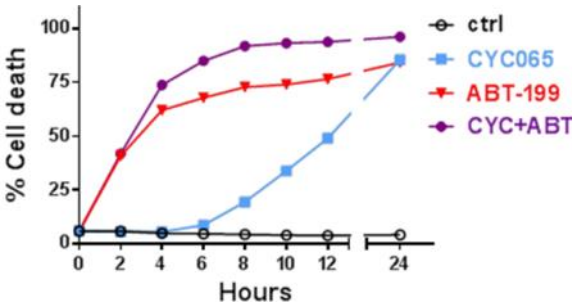

Figure 1: Synergistic cell killing activity of CYC065 and ABT-199 (venetoclax) in primary CLL model[2]

Mcl-1 dependent cancers

Mcl-1 is overexpressed in many types of cancer acting as a survival and drug resistance mechanism. Multiple studies show that knockdown of Mcl-1 leads to cancer cell death and resensitization to drug treatment.[3,4]

Chronic lymphocytic leukemia (CLL) cell survival depends on the expression of anti-apoptotic proteins, including Mcl-1 and Bcl-2. In this context, targeting Mcl-1 or Bcl-1 releases pro-death signals and commits CLL cells to apoptosis. Venetoclax was recently approved as a second line treatment of relapsed/refractory CLL with or without 17p deletion after at least one prior therapy.

The pan-CDK inhibitors flavopiridol and dinaciclib have shown efficacy in CLL clinical trials, providing clinical proof-of-concept for the targeting of anti-apoptotic pathways in such leukemias. Mcl-1 expression can modulate resistance to Bcl-2 inhibition and is known to be upregulated in lymph node CLL cells, possibly leading to resistance to venetoclax.

Rapid and complete cell death was induced in **CLL** and **multiple myeloma** cell lines after short exposure to CYC065 in the presence of stromal cells which confer protection from standard treatments.[5,6] Mcl-1 down-regulation was observed, consistent with the pro-apoptotic mechanism of CYC065. CYC065 synergizes with venetoclax in preclinical models at clinically achievable concentrations, supporting clinical investigation of combination regimens of CYC065 and venetoclax.[7,8,9]

Drug resistance in **AML** has been attributed among others to high levels of Mcl-1. AML cell lines are highly sensitive to CYC065[9] and 5 to 8 hours treatment is sufficient to achieve induction of cell death. CYC065 has single agent efficacy in AML xenografts and the potential to be combined with approved AML therapies. In leukemia cells harboring the rearranged Mixed Lineage Leukemia gene (MLLr), CYC065 reduced both Mcl-1 expression and CDK9 dependent transcription of MLL-regulated leukemogenic genes.[10]

MYC-addicted cancers

MYC proto-oncogenes encode MYC family proteins which are overexpressed in over 50% of human cancers often via gene amplification. MYC proteins are transcriptional regulators which promote cancer cell growth and survival by increasing the expression of target genes involved in cell metabolism and growth. MYCN gene amplification is found in 45% of high-risk neuroblastomas (NB), a childhood cancer with <10% long term survival. CDK9 mediates transcriptional regulation of MYCN.

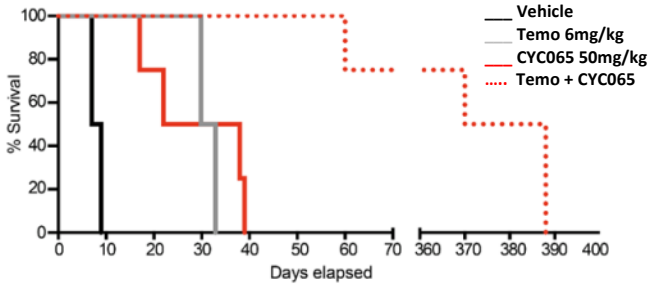

Figure 2: CYC065 prolongs survival in Th-MYCN neuroblastoma model

NB cell lines with MYCN amplification are highly sensitive to CYC065. CYC065 transcriptionally downregulates MYCN expression causing tumor regression in an aggressive genetic model of MYCN driven cancer and prolongs survival in MYCN-addicted NB xenografts.[11] CYC065 may have therapeutic potential in NB and other cancers where MYC overexpression is implicated in tumorigenesis or drug resistance. CYC065 has been shown to reduce MYC expression among others in B-cell lymphoma and triple negative breast cancer.[7]

Reversal of CDK2/cyclin E mediated drug resistance

Preclinical and clinical evidence shows that CDK2/cyclin E activation or cyclin E (aka CCNE) overexpression is a mechanism of drug resistance in metastatic breast cancer. This has been shown for HER2+, triple negative and ER+ breast and high grade serous ovarian cancers, as resistance to trastuzumab, AI, AI plus CDK4/6 combinations, and chemotherapy respectively. CYC065 resensitized trastuzumab-resistant cells to apoptotic cell killing, was effective against uterine serous carcinomas (USC) with amplified/overexpressed cyclin E and was synergistic with PIK3CA inhibition *in vivo*.[12,13,14]

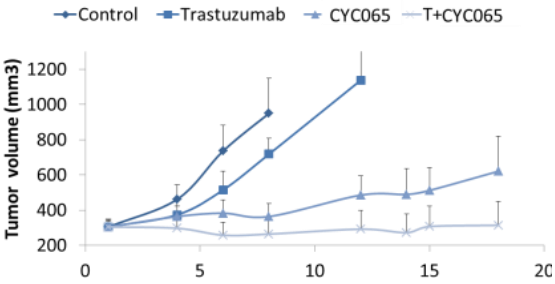

Figure 3: CYC065 Activity in HER2+, Cyclin E-addicted, Trastuzumab-resistant Breast Cancer

Development Status

In 26 patients treated with CYC065 as a 4-hour infusion once every 3 weeks RP2D was 192 mg/m^2. Mcl-1 suppression lasting at least 24 hours was observed in 11 of 13 evaluable patients treated at the RP2D. Stable disease ≥ 6 cycles was observed in 6 patients of which 4 had molecular features associated with CYC065's mechanism, including Mcl-1, MYC or cyclin E. Two ongoing patients with uterine and ovarian cancer received 9 and 17 cycles respectively. DLTs are reversible neutropenia, thrombocytopenia, febrile neutropenia, diarrhea, hypomagnesemia, white blood cell lysis syndrome and its associated electrolyte abnormalities and liver enzyme elevations. Part 2 of the study will evaluate additional dosing schedules in patients with advanced solid tumors, in particular those with amplification of Mcl-1, MYC or cyclin E. CYC065 will also be evaluated as a single agent in MDS/advanced leukemia patients. Studies in other mechanistically relevant cancers are being planned.

Endnotes:

1. *An experimental drug under clinical investigation. Not approved for human use. Mcl-1 = myeloid cell leukemia protein, a BCL-2 family apoptosis regulator that enhances cancer cell survival by inhibiting apoptosis.*
2. *Rong, et al, AACR 2017 Abs 5095*
3. *Caldon, et al, Mol. Cancer Ther. 2012, 11:1488*
4. *Quinn et al 2011 Expert Opin. Investig. Drugs 20:1397*
5. *Chen, et al, AACR 2010 Abs 4431*
6. *Pozzi, et al, 2010 ASH Ann Meet Abs 2999*
7. *MacKay, et al, AACR-NCI-EORTC 2015 Abs 182*
8. *Frame et al_AACR 2016_Abs 1309*
9. *Zheleva, et al, SOHO 2015 Abs 213*
10. *Chen et al, AACR 2018 Abs 3905/5*
11. *Poon E et al Childhood Cancer Meeting 2016, September 5 – 7th, London, UK, Abs. 1-19*
12. *Scaltriti, et al, 2011 PNAS 108:3761*
13. *Cocco, et al, 2016 BJC 115:303-311*
14. *Akli, et al, 2011 Cancer Res 71:3377*

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