A unique blood-based biomarker - Thymidine Kinase activity - reflects cell proliferation and treatment efficacy in oncology drug development and clinical studies

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DiviTum® TKa

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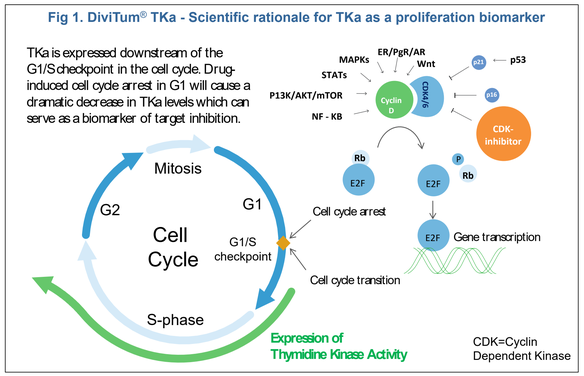
Background and Rationale

- Thymidine kinase (TK) is an enzyme that plays a key role in DNA replication. The expression of TK is tightly linked to the cell cycle, and its presence or absence can serve as a biomarker of active cell proliferation - "a liquid Ki67".
- TK activity (TKa) can provide an early indication and real-time data of the biologic activity of oncology drugs in both preclinical and clinical trials. DiviTum® TKa is an ELISA based assay that can accurately measure TKa in blood samples and/or cell extracts. The DiviTum® TKa assay is FDA cleared for clinical use.
- FDA's Project Optimus initiative: TKa can serve as a pharmacodynamic biomarker for drug effects on tumor proliferation. Incorporating TKa assessment into drug dose optimization studies can provide information about minimally effective dose selection to address the FDA's Project Optimus initiative.

Fig 1. DiviTum® TKa - Scientific rationale for TKa as a proliferation biomarker



Objective

To illustrate the utility of TKa as a biomarker for oncology drug dose response and treatment efficacy in both preclinical and clinical contexts. CDK4/6 inhibitors and Immune Checkpoint Inhibitors (ICIs) are provided as examples.

Method

Cell culture: TKa response to increasing concentrations of palbociclib treatment for 6 hours was determined by DiviTum® TKa analysis of lysed K562 myelogenous leukemia cell extracts. Cell viability was determined by Trypan blue staining.
Mouse models: Human breast cancer MCF-7 cells were implanted into a right lower mammary gland in NOD-SCID (NOG), 7-9 weeks old, female mice. Palbociclib was injected via gavage in 50 mM lactate buffer (pH4) 4 times at 24h intervals. Sacrifice was 4h after the last injection. Murine breast cancer E0771 cells: Procedure as for MCF7 except that the animals used were C57BL6, 6-7 weeks old, female mice.
Clinical studies: Circulating TKa was measured at different time points in serum or plasma samples from patients with metastatic breast cancer (MBC) treated with endocrine therapy (ET) + CDK4/6 inhibitors. Metastatic malignant melanoma patients treated with ICI's. TKa was measured with the DiviTum® TKa assay.

Method (cont'd)

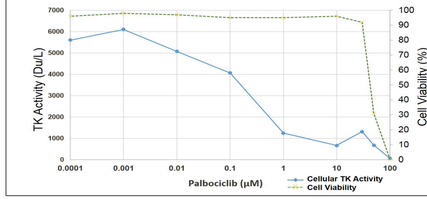
Postmenopausal patients diagnosed with hormone receptor positive (HR+) MBC treated with endocrine therapy + CDK4/6i had serum collected at baseline (screening), at cycle 1 day 15 and cycle 2 day 1 (day 28).

Patients with unresectable, metastatic malignant melanoma treated with ICI (anti-PD-1 and/or anti-CTLA-4) had plasma collected at baseline, one month into treatment and at end of treatment/follow-up.

Cell Study Results

Cultured K562 cells were treated with palbociclib and harvested after 6 h. Viability and intracellular TKa were determined at time of harvest.

Fig 2. Dose dependent reduction of TK activity in response to CDK4/6 Inhibition



TKa was reduced in a linear and dose-dependent manner in response to palbociclib.

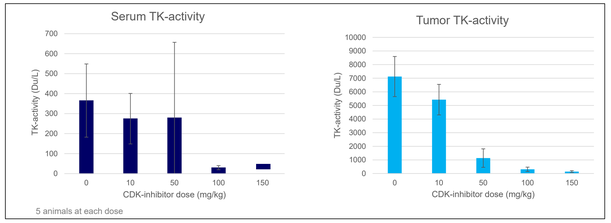
TKa provides evidence of palbociclib effects on cell proliferation at doses where viability is unaffected.

Using TKa to measure the impact on cell proliferation as a drug evaluation endpoint can confirm mechanism of action efficacy and can help inform a minimally effective dose for a new compound rather than a maximally tolerated dose. In both cultured cells and mouse xenografts, an effect on tumor cell proliferation (assessed by TKa) can be observed well before and at much lower doses than when cell death or toxicity is used as the dose determination endpoint.

Mouse Study Results

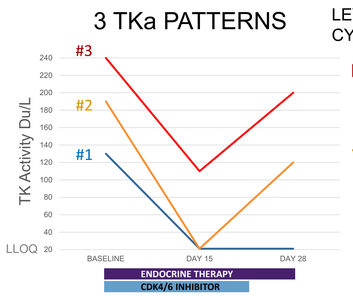
In mice grafted with MCF-7 human breast cancer cells, both the serum TKa and intracellular tumor TKa were reduced in a dose dependent manner in response to palbociclib (fig 3). TKa provides evidence of efficacy of CDK4/6 inhibition on cell proliferation.

Fig 3. TKa in serum and tumor extracts from mice given different doses of palbociclib



Clinical Study Results CDK4/6i's

Fig 4. TKa patterns predict duration of CDK4/6i therapy in HR+ MBC patients



Patients achieving a complete TKa suppression two weeks into CDK4/6i treatment, sustained at 4 weeks of treatment, have a long duration on therapy with very few patients progressing in the first 12 months when treated in the 1st line (Fig 4. Pattern #1). In contrast, patients whose TKa levels never fall below the LLOQ (Lower Limit of Quantification) of the DiviTum® TKa assay have a much worse outcome with a median PFS of 10.1 months with 1st line CDK4/6i therapy (Pattern #3).

Fig 5. Three patterns of TKa in serum from three MBC studies of ET+CDK4/6i's

	BioltaLEE R+L 1st line	PalboAlt P+L/F 1st line	PYTHIA P+F 2nd line
Pattern 3	10 mo	4 mo	5 mo
Pattern 2	22 mo	21 mo	13 mo
Pattern 1	NR	34 mo	17 mo
Patients (n)	287	54	124

R=ribociclib
P=palbociclib
L=letrozole
F=fulvestrant

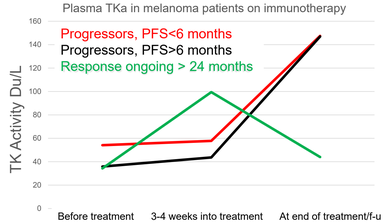
Median PFS in months (mo).

Pattern 3: Incomplete suppression of TKa at Day 15 and Day 28
Pattern 2: Complete suppression of TKa at Day 15, rebound >assay LLOQ at Day 28
Pattern 1: Complete suppression of TKa at Day 15 and Day 28
HR+ MBC patient levels of serum TKa at baseline (screening), Day 15 and Day 28 during first cycle of endocrine therapy and CDK4/6i. Levels below LLOQ of the DiviTum® TKa assay set as complete suppression of TKa/cell cycle arrest.

In total, 6 clinical HR+ MBC studies have presented results that support TKa as a biomarker of early prediction of patient response to CDK4/6 inhibition. TKa can be used as a readout for successful drug target engagement, for biomarker-driven patient selection and enrichment in drug development clinical trials.

Clinical Study Results ICI's

Fig 6. TKa dynamics in metastatic malignant melanoma patients treated with ICI's



3 different patterns of TKa in association with ICI treatment in patients with metastatic melanoma; before ICI treatment start, 3-4 weeks into treatment and at end of treatment/follow-up. Median TKa values at the three different time points.

When effective, ICIs cause an increase in immune cell proliferation which can be detected by elevated/high levels of TKa. Patients who do achieve an early, on-treatment TKa level increase (>60 Du/L, >1.5 fold) as compared to BL, have a significantly longer PFS (HR=2.47, p<0.05) and patients with a high TKa during ICI treatment have significantly longer OS (HR=3.49, p<0.05).

- Low pretreatment TKa levels are associated with superior ICI treatment efficacy.
- Increased TKa levels early into ICI treatment can be predictive for long PFS and OS.
- The TKa rise could be related to either TKa release from dying cells or from proliferating immune cells resulting from the ICIs stimulation, or possibly both.

Conclusions

- **TKa is a translational liquid biomarker that can bridge between preclinical and clinical studies, providing fundamental information for candidate selection, dosing and protocol design during early and late-stage drug development.**
- **The DiviTum® TKa assay is a useful tool when evaluating the efficacy of drug candidates that target the cell cycle. It can also be helpful for PK/PD modeling.**
- **The FDA cleared DiviTum® TKa assay provides early evidence of treatment response and resistance to new drugs in development including cell cycle- and Immune Checkpoint Inhibitors. It can be used for patient selection and can help inform minimally effective dose determination to facilitate FDA Project Optimus goals.**

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