Evaluation of TK1 protein changes in Acute Myeloid Leukemia (AML) xenograft model during 5-FU treatment using the AroCell TK 210 ELISA

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Background

- Thymidine Kinase 1 (TK1) is a cytosolic enzyme that phosphorylates deoxy thymidine to deoxy thymidine monophosphate (dTMP) using ATP as the phosphate donor. dTMP undergoes further phosphorylation and is incorporated into DNA during the S phase of cell cycle (1).
- During un controlled proliferation, TK1 leaks from the damaged and dying cells into the blood where it forms stable aggregates (2).
- The strong association of TK1 with DNA precursor synthesis has made serum TK1 an established biomarker for the prognosis and therapy monitoring of various malignancies (3).
- However, most of the commercial TK activity assays have limitations for their use in xenograft models as they cross react with TK1 from the host species.
- To overcome these limitations, AroCell developed a sandwich ELISA assay for the quantification of TK1 protein levels (4).
- Recent studies have shown that AroCell TK 210 ELISA can be a valuable tool for evaluating the effect of anti-cancer drugs in cell lines (5).
- 5-Flurouracil (5-FU) is a pyrimidine analog and it is the most widely used chemo-therapeutic agent for treating various cancer diseases.
- 5-FU blocks the de-novo synthesis of dTMP and so favors the survival of cells with a high expression of TK1 (6).
- In this study, we evaluated the changes in TK1 protein levels in an acute myeloid leukemia (AML) xenograft model during and after treatment with 5-FU.

Materials and Methods

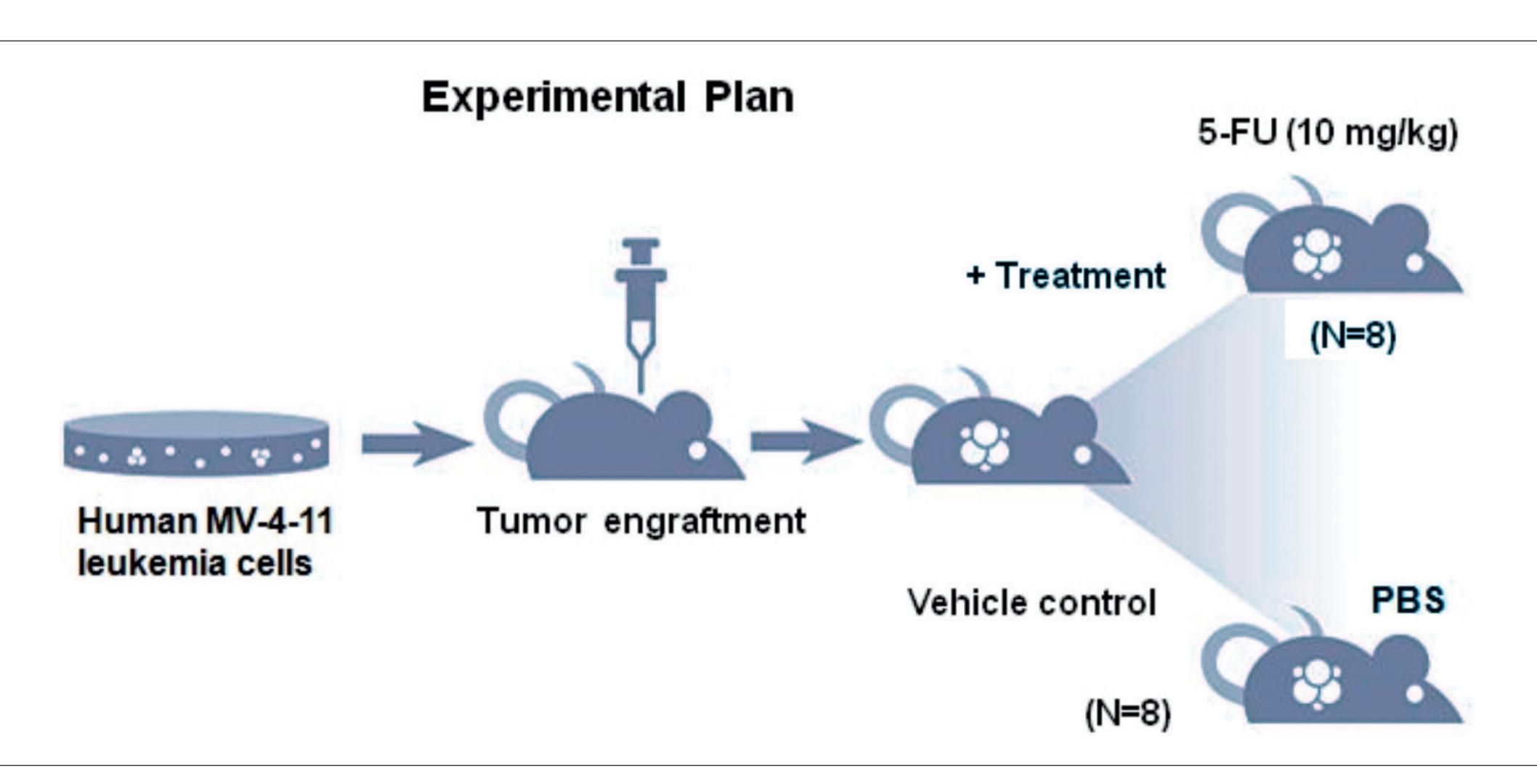


Figure 1: AML xenograft model experimental set up

- Human MV-4-11 (ATCC® CRL9591™) leukemic cells from ATCC were cultured with 95% O₂ and 5% CO₃ at 37°C in IMD medium (Sigma) supplemented with 10% FBS (HI media) containing 4mmol/L L-glutamine, 5 ng/mL GM-CSF and 1% penicillin and streptomycin.
- Athymic BALB/c nude mice in the age range of 8-10 weeks were divided into vehicle controls (n=8) and treatment (n=8) groups (Fig 1).
- Five million cells in 0.2 mL of serum free medium with 50% Matrigel were injected into the left flank of each mouse.
- The tumors were allowed to grow until the average tumour volume reached 50-100 mm3.
- Then the treatment group received 5-FU (10 mg/kg, once daily), while the control group received vehicle control (PBS) for three weeks.
- Tumor volumes were measured every 3rd day during the whole period of the treatment in both control and treated mice.
- Serum samples (70 μL) were collected on day 1 after treatment initiation, day 7, day 13, day 21 and on the day of termination (day 25).
- All the Xenograft model experiments were performed at TheraIndx life sciences Pvt Ltd, Bangalore, India as per CPCSEA guidelines. Samples were stored at -20°C until further analysis.
- TK1 protein levels in serum samples were determined in both the control and treated mice groups using the AroCell TK 210 ELISA. The AroCell TK 210 ELISA assay was performed according to IFU as summarized in Fig 2 (www.arocell.com).

AroCell TK210 ELISA: A Sensitive Assay to Measure the TK1 Protein

STEP 1: Pre-incubation

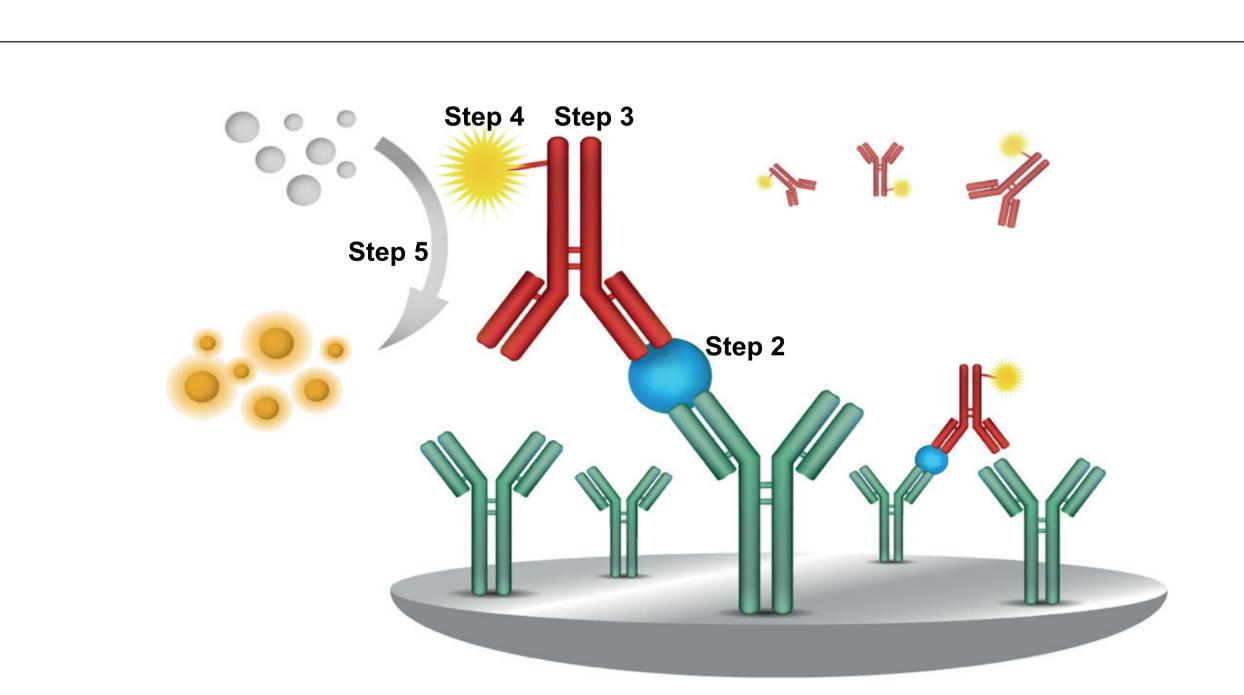
Samples (serum/plasma) are pre-incubated with AroCell sample dilution buffer for 1 hour, the buffer will expose the TK1 epitope that fecilitates the binding of antibody to TK1 in samples.

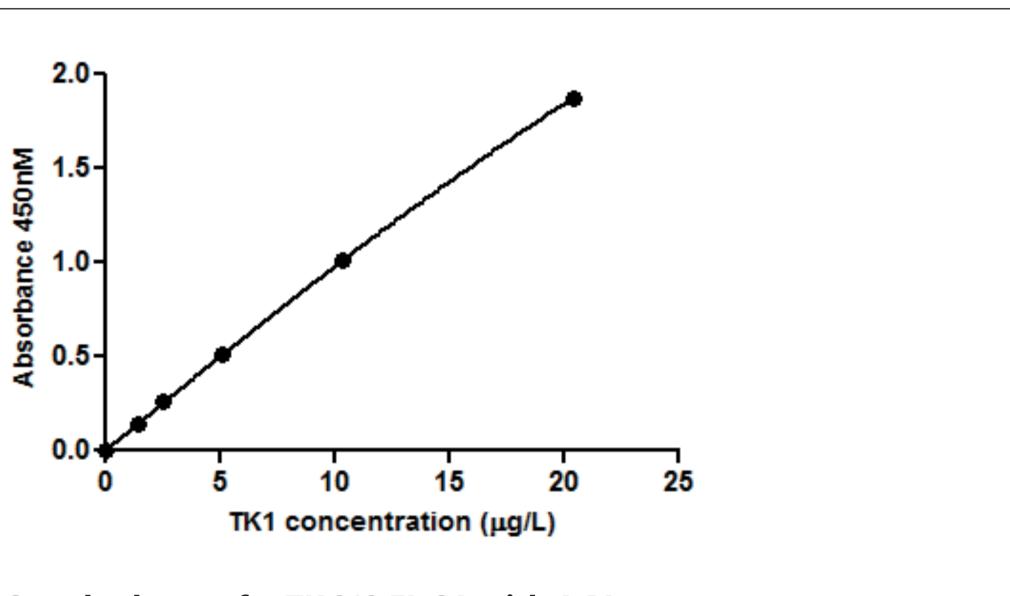
STEP 2: Addition of pre-incubated samples to antibody coated plate.

STEP 3: Detection with biotinlyated TK1 antibody.

STEP 4: Streptavidin-HRP conjugate was added to detect the biotinlyated antibody bound complex.

STEP 5: Addition of TMB substrate produces the optical density (O.D) of the colour. TK1 protein levels in samples were calculated by using the standard curve with 4PL curve fit.





Standard curve for TK 210 ELISA with 4-PL (absorbance of calibrators reduced from the blank)

Figure 2. Principle of the TK 210 ELISA

Results

- Overall, there was a significant difference (p<0.05) in the mean tumour volumes between 5-FU treated (5-FU) (108.75± 36.3 mm³) and untreated control mice (294.63± 50.17 mm³) at the end of treatment period.
- A significant reduction in the tumor volume was observed from the 2nd week on words in 5-FU treated mice up to the termination (25 days) compared to control mice (Table 1).

	Tumour volume (mm3) [Mean± SEM]						
Group	Day 1	Day 7	Day 13	Day 21	Day 25		
Control mice	73.5 ± 21.8	93.3 ± 25.0	117.6 ± 26.9	183.9 ± 30.6	294.6 ± 50.2		
5-FU treated mice	65.5 ± 22.9	60 ± 24.7	70.5 ± 26.2*	85.3 ± 29.1*	108.75 ± 36.3*		
* C:: C							

* Significantly different from control (P<0.05)

Table 1: Change in the tumour volumes of untreated and 5-FU treated mice

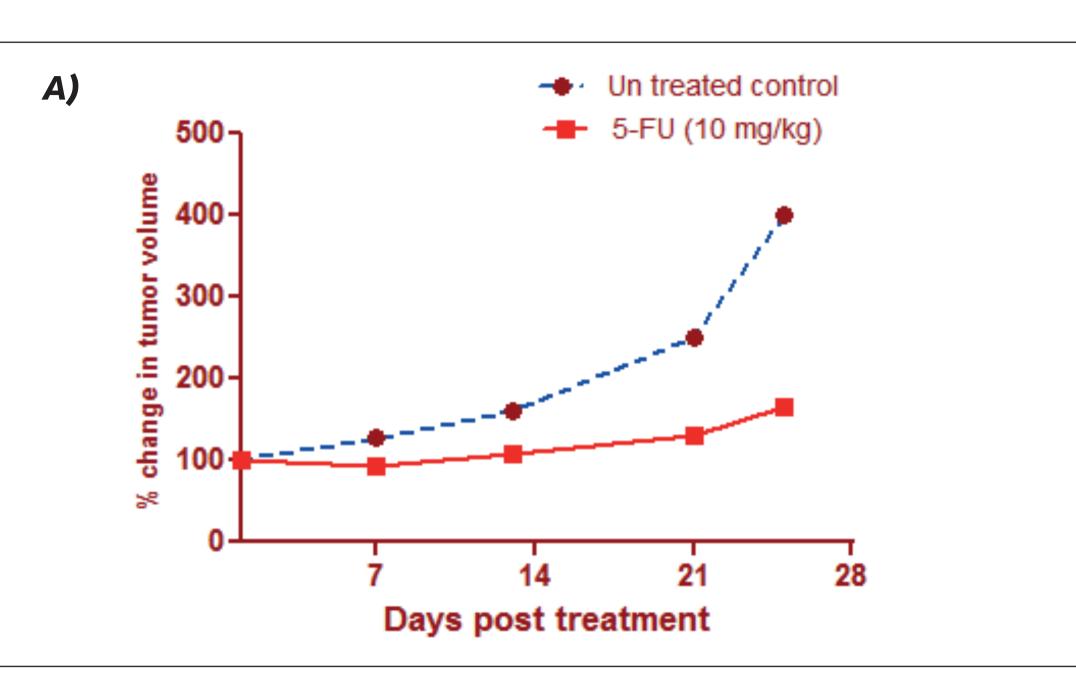
• The serum TK1 protein levels in the control mice increased as the tumour volume progressed (0.1 to 0.22 μg/L). Similarly, the TK1 protein levels increased significantly, but to a greater degree, in the 5-FU treated mice (0.18 to 0.45 μg/L) on days 13, 21 and 25 compared to control mice (Table 2).

	TK1 protein (μ	TK1 protein (μg/L) [Mean± SEM]					
Group	Day 1	Day 7	Day 13	Day 21	Day 25		
Control mice	0.1 ± 0.001	0.1 ± 0.016	0.1 ± 0.02	0.13 ± 0.05	0.22 ± 0.025		
5-FU treated mice	0.18 ± 0.04	0.3 ± 0.042	0.42 ± 0.19*	0.45 ± 0.15*	0.43 ± 0.08*		

^{*} Significantly different from control (P<0.05)

Table 2. Change in the serum TK1 protein levels in control and 5-FU treated mice.

- The changes in tumor volume and TK1 protein levels during treatment were calculated by using the day 1 value for normalization.
- The tumor volume increased up to 4 fold by day 25 in the controls (Fig 3A). Whereas in the 5-FU treated mice, the change in tumor volume was maximally 1.5 fold compared to day 1.
- These results showed that 5-FU treatment had significantly reduced tumor growth compared to control mice.



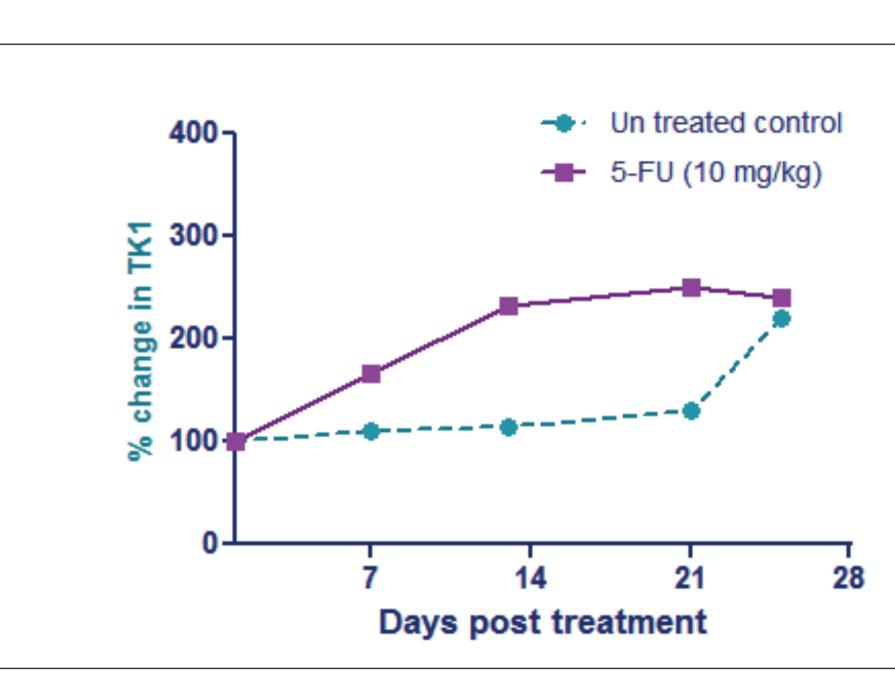
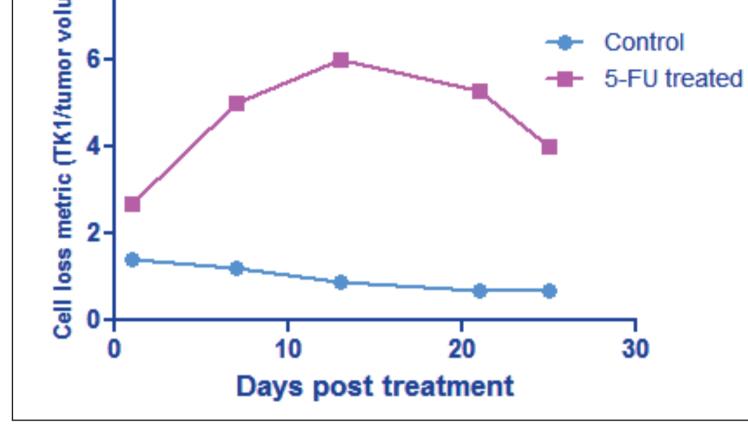


Figure 3. % change in the (A) tumor volumes and (B) TK1 protein evels in control and 5-FU treated mice

- In 5-FU treated mice, the serum TK1 protein concentration increased more than 2.5 fold on day 21 compared to day 1. In controls, the concentration was little changed (Fig 3B).
- 5-FU blocks the de-novo thymidine synthetic pathway leading to up-regulation of the salvage pathway which, together with increased cell death, resulted in high TK1 levels (6).
- In control animals, prior to day 21 serum TK1 levels increased to a lesser degree than in 5-FU treated mice. The increase between days 21 and 25 could be due to the rapid tumor growth during this period.
- Based on serum TK1 protein and tumour volume, a cell loss metric was calculated (CLM).
- CLM = TK1 concentration ($\mu g/L$) / Tumor volume (mm³).
- CLM provides information about the rate of cell turnover.
- In 5-FU treated mice, the CLM was 2 fold higher after 2 weeks of treatment compared to day 1 (Fig 4). This indicates that the tumor cells' response to the drug resulted in increased release of TK1 into the blood.



• However, in control animals the CLM was low throughout the study period suggesting that tumor cell proliferation in this tumor model is not associated with increased cancer cell turnover. Figure 4. Cell loss metric in Xenograft models

CONCLUSIONS

- Serum TK1 protein levels were increased significantly in 5-FU treated mice compared to untreated control mice, due to increased levels and release of TK1 in response to blocking of the de-novo dTMP synthetic pathway by 5-FU.
- The main finding in this study is that the changes in TK1 protein levels measured with AroCell TK 210 ELISA were significant and should be sufficient to detect a drug response in the AML xenograft mice model after the 2nd week of treatment.
- These preliminary results will widen the application of the AroCell TK 210 ELISA as a translational biomarker in drug development.

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Acknowledgements:

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