# The cytotoxic effect of $^{64}$Cu/NOTA-terpyridine platinum conjugate, as a novel chemoradiotherapy (CRT) agent

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## INTRODUCTION:

Colorectal cancer is one of the most prevalent cancers worldwide that displays both intrinsic and acquired resistance to platinum-based chemotherapeutic agents (Pt-CAs). To overcome such resistance new classes of Pt-CAs have been proposed, including terpyridine (TP) compounds that targets the G-quadruplex tertiary structure of DNA. Additionally, recent studies indicate a maximum chemoradiation benefit, when radiation is administered with Pt-CAs at their highest concentrations in cancer cell DNA. Accordingly, we synthesized a novel chemoradiotheranostic (CRT) agent by conjugating a TP moiety with $^{64}$Cu ($^{64}$Cu-NOTA-TP).

## METHODS:

The in-vitro cytotoxic and synergistic effects of complexes were assessed by Presto-blue assay. The cellular uptake, internalization and efflux of $^{64}$Cu-NOTA terpyridine platinum complex was measured for colorectal cancer cell (HCT116) as well as a normal fibroblast cell line (GM05757) at 24, 48 and 72 hours after initial incubation time.

### Prestoblue Assay

Seed 5000 cells in a 96-well culture plate for 24h

Media was wasted and the cells were washed with PBS twice. platinum compounds diluted in media was added to cells for 8, 24, 48, 72h

Once the time course of experimental conditions has been completed, add stain to the culture media

Incubate for 10 min at 37 °C

Read by fluorescence or absorbance (Excitation:550nm, Emission:590nm)

### Internalization Assay

Seed 2 × $10^5$ cells in a 12-well culture plate for 48h

The cells were washed thrice with PBS, then 950 µL of culture medium was added. HCT116 cells were incubated 15min to 72h with 111 kBq (3 µCi; 50 µL)

To collect surface-bound drugs, the cells were washed with acidic buffer (Glycine-HCl, 50mM, PH=2.2)

Then, the cells were washed with PBS. The cells were harvested and counted in a gamma counter

The results are expressed as percentage of added dose retained per $10^6$cells (%AD/$10^6$ cells)

## RESULTS :

- $^{cold}$Cu-labeled NOTA-terpyridine platinum complex showed 3.4, 1.7 and 2.3 times higher cytotoxicity against HCT116 cells relative to GM05757 fibroblast normal cells (table1, entry2).
- Radiolabelling NOTA-TP with $^{64}$Cu resulted in 17530-, 40083- and 66000-fold enhancements in its cytotoxicity against HCT116 cells ($EC_{50}$=0.017±0.004, 0.012±0.006 and 0.005±0.002µM) as compared to $^{cold}$Cu-NOTA-terpyridine ($EC_{50}$ = 298 ± 2, 481 ± 25 and 330 ± 51µM) at 24, 48 and 72h post-administration, respectively (table1, entry4).
- The cytotoxicity of the $^{64}$Cu-conjugate toward HCT116 cells was about 3.8-fold higher than that of GM05757 cells at 24 and 72h. This result was consistent with a 2-3-fold higher internalization of $^{64}$Cu-conjugate in HCT116 cells relative to GM05757 cells at similar times (figure2, E). The internalized activity of the $^{64}$Cu-conjugate steadily increased from 0.04±0.02% to 18.7±2.8% over 24h incubation time (figure2, B).
- Efflux kinetics of the $^{64}$Cu-conjugate showed that more than 40% of internalized activity was retained by cancer cells over a 24h (figure2, C).

## RESULTS :

**Table 1** $EC_{50}$ values (µM) of platinum compounds for both cancer HCT116 cells and normal fibroblast GM05757

| | | 24h | | 48h | | 72h | |
|---|---|---|---|---|---|---|---|
| **Entry** | **Compounds** | **GM05757** | **HCT116** | **GM05757** | **HCT116** | **GM05757** | **HCT116** |
| **1** | **NOTA-TPt** | 504 ± 4 | > 700a | 202 ± 5 | 63 ± 2 | 51 ± 3 | 24 ± 1[a] |
| **2** | **$^{cold}$Cu-NOTA-TPt** | > 1000 | 298 ± 2 | 839 ± 2 | 481 ± 25 | 747 ± 26 | 330 ± 51 |
| **3** | **$^{64}$Cu-NOTA-TPt[b]** | >200 | 59 ± 3 | N/A | 9±2 | 12±2 | <5 |
| **4** | **$^{64}$Cu-NOTA-TPt[c]** | >0.066 | 0.017±0.004 | 0.025±0.005 | 0.012±0.006 | 0.019±0.004 | 0.005±0.002 |
| **5** | **Cisplatin** | 88 ± 4 | 31 ± 2 | 84 ± 2 | 42 ± 8 | 77 ± 1 | 23 ± 3 |
| **6** | **Oxaliplatin** | > 200 | > 200 | 165 ± 9 | 64 ± 1 | 65 ± 3 | 16 ± 4 |

[a]significance level between 72 h and 24 h (P <0.01) time points; [b] The apparent molar activity of the [$^{64}$Cu]Cu-NOTA-TPt solution was ranged from 0.84 to 4 MBq/nmol. [c] The apparent molar activity of the [$^{64}$Cu]Cu-NOTA-TPt solution was 119MBq/nmol.

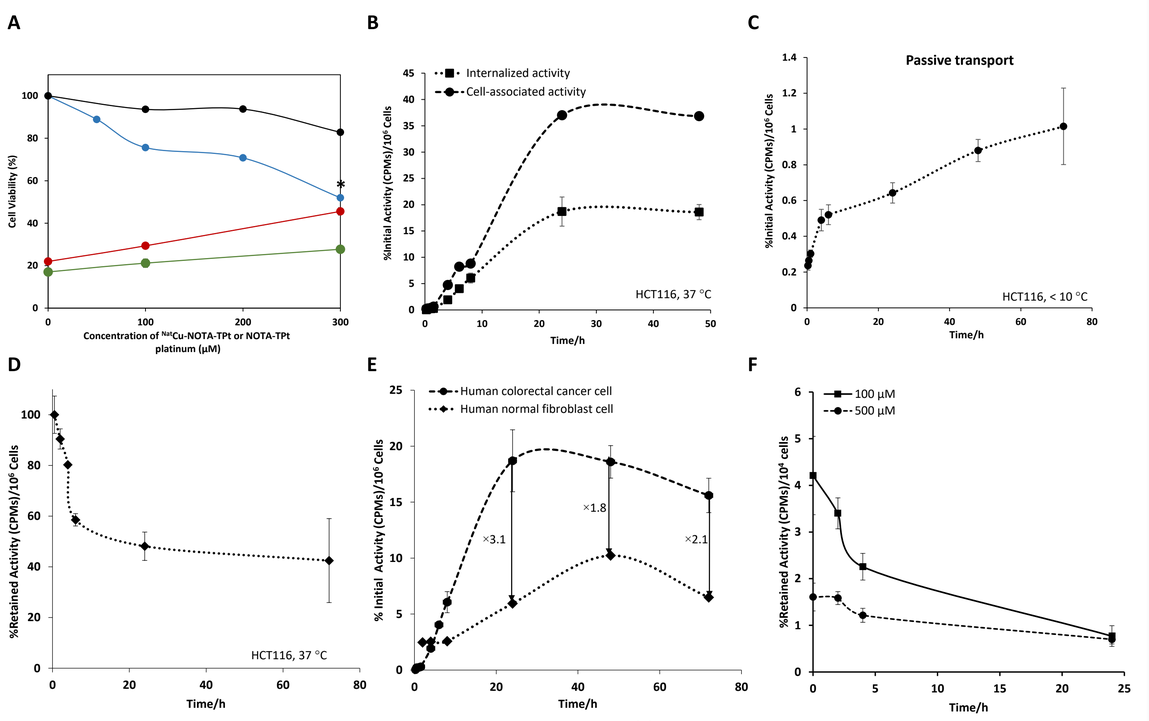


**Fig 2:** Cell viability of NOTA-TPt (black line), $^{Cold}$Cu-NOTA-TPt (blue line) and $^{64}$Cu-NOTA-TPt at 5 MBq (red line) and 8 MBq (green line) measured after 24 h incubation in HCT116 cells. *Significant difference (P <0.05) of cell viability compared to $^{cold}$Cu-NOTA-TPt alone **(Frame A)**. The percentages of total cell-associated and internalized at 37 °C **(Frame B)** and < 10 °C **(Frame C)**. Cell efflux assay from HCT116 cells after 1h incubation with $^{64}$Cu-NOTA-terpyridine Platinum compound over a 72 h period, data are presented as total activity (%)/$10^6$ cells **(Frame D).** The internalized fraction of $^{64}$Cu-conjugate in both cancer (HCT116) and normal fibroblast (GM05757) cells **(Frame E)**. Percentage of retained activity of $^{64}$Cu/NOTA-Terpyridine Platinum in HCT116 cell lines over a 24h time course. The ratios are expressed as the percentage of retained activity per initial loaded activity. Data are presented as total activity (%)/$10^4$ cells **(Frame F).**

## CONCLUSION :

In conclusion, these results supports the potential use of $^{64}$Cu-labeled terpyridine platinum complex as a novel CRT agent to diagnose and treat cancers.

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