# Ruthenium-based agents as promising metallodrugs to fight colorectal cancer


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## BACKGROUND

**Colorectal cancer** (CRC) is one of the most lethal cancers worldwide[1]. CRC therapy has **limited chemotherapeutic agents available[2]**. **Ruthenium (Ru) drugs** have arisen as one of the most promising metallodrugs with features that increase their specificity and selectivity toward cancer cells[3]. In this work, we studied **three new Ru-cyclopentadienyl (RuCp) conjugates** (**LCR134, PMC78** and **LCR220**) in CRC cells (**Figure 1**). These conjugates comprise a **ruthenium cytotoxic core** with anticancer properties (**LCR134, PMC78** and **LCR220**), a **biodegradable polymer chain** (**PMC78** and **LCR220**) allowing the accumulation by the enhanced permeability and retention effect (EPR), and a **biotin molecule** (**LCR134** and **LCR220**) allowing a selective accumulation in malignant tissues[4,5]. The addition of **biotin** to the **ruthenium cytotoxic core** also constitutes an approach to **increase the targeting to cancer cells**, due to the **overexpression of vitamin receptors** in these cells. The conjugation of these characteristics make these complexes more specific, efficient and potentially selective to target cancer cells.

## AIM

**To unveil the effect and mechanism of action of RuCp agents in CRC cells harboring different oncogenic mutations (SW480$^{KRAS}$, RKO$^{BRAF}$) in comparison to normal colon cells (NCM460).**

## RESULTS

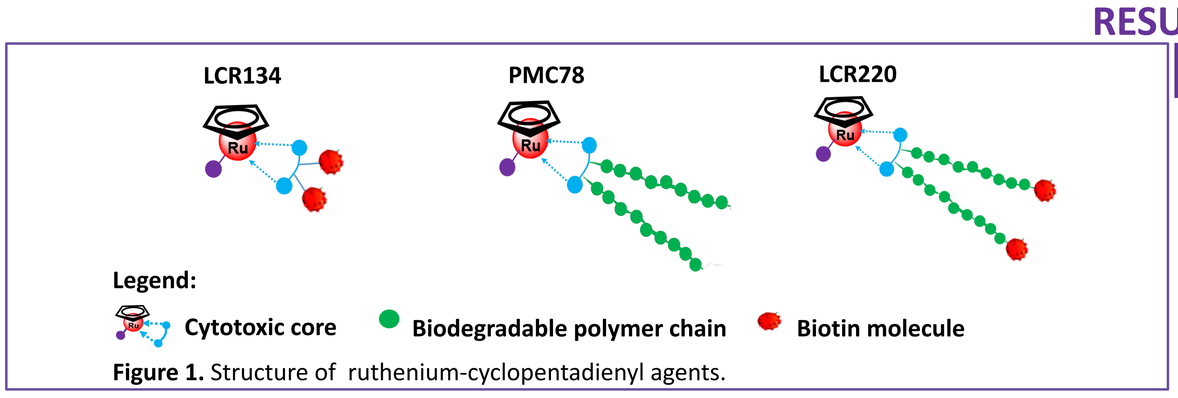


**Figure 1.** Structure of ruthenium-cyclopentadienyl agents.

### Ru agents are more cytotoxic and potentially selective to CRC cells

**Table 1.** Determination of the $IC_{50}$ values of Ru agents by Sulforhodamine B.

| | SW480$^{KRAS}$ | RKO$^{BRAF}$ | NCM460 | Selectivity Index | |
|---|---|---|---|---|---|
| **Compound** | $IC_{50}$ (μM) | $IC_{50}$ (μM) | $IC_{50}$ (μM) | SW480 | RKO |
| **LCR134** | 14,1 | 7,7 | 57,2 | 4,1 | 7,4 |
| **PMC78** | 6,0 | 4,0 | 15,0 | 2,5 | 3,8 |
| **LCR220** | 1,8 | 2,8 | 4,3 | 2,4 | 1,5 |

### Ru agents decrease the clonogenic ability of CRC cells

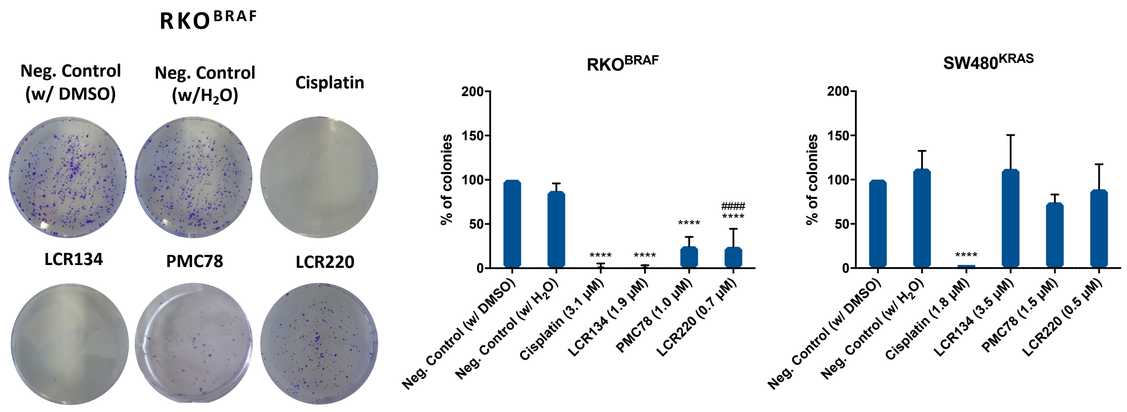


**Figure 2.** Representative images of RKO and graphics of colony formation ability of RKO and SW480 cells.

### Ru agents induce alterations in cell cycle of CRC cells

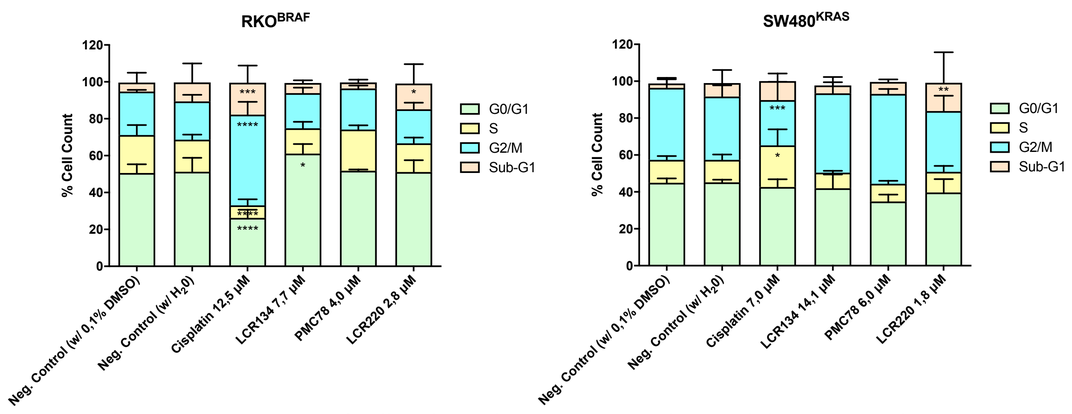


**Figure 3.** Graphical representation of cell cycle analysis using propidium iodide in RKO and SW480 cells.

### Ru agents induce apoptosis at high concentrations in CRC cells

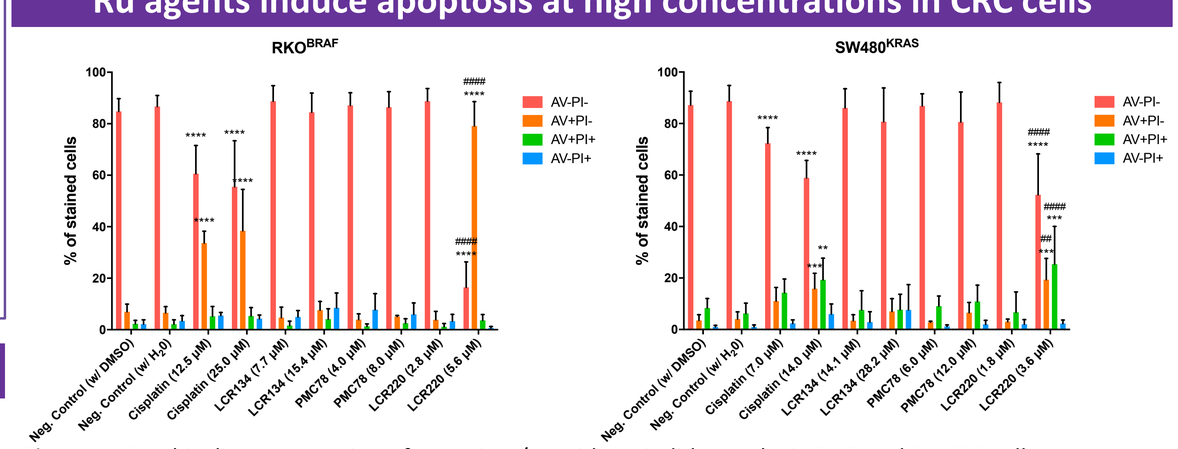


**Figure 4.** Graphical representation of annexin V/propidium iodide results in RKO and SW480 cells.

### Ru agents affect cell migration in CRC cells

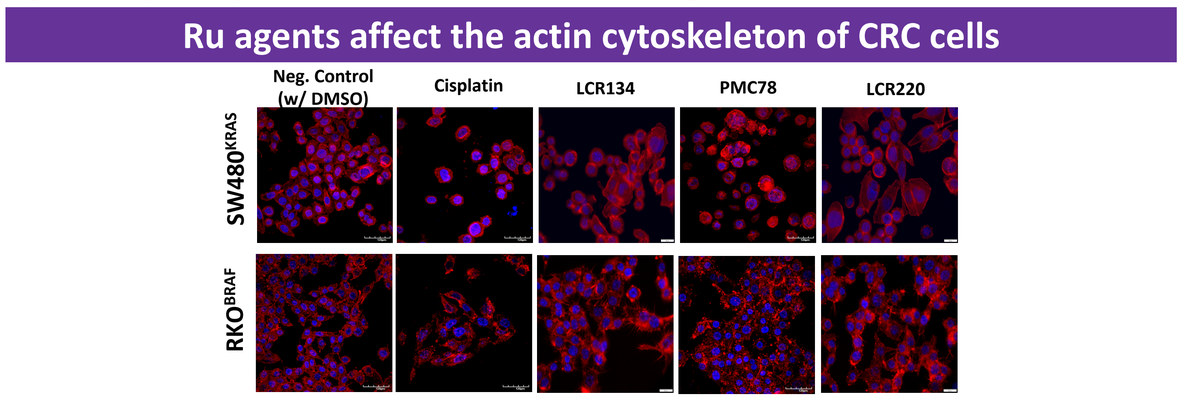


**Figure 5.** Representative images of RKO cells and graphical representation of wound healing assay in RKO and SW480 cells.

### Ru agents are located in cytoskeleton and membrane in CRC cells

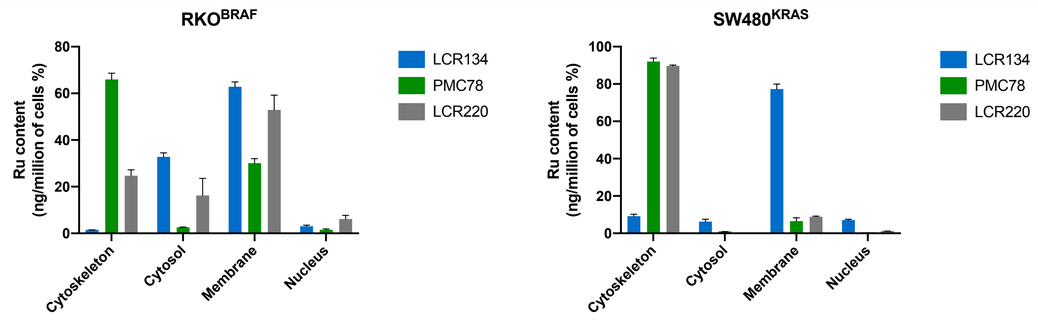


**Figure 6.** Graphical representation of cellular distribution of Ru agents in RKO and SW480 cells.

### Ru agents affect the actin cytoskeleton of CRC cells




**Figure 7.** Analysis of F-actin staining in CRC cells. Representative images (×600) of DAPI, Phalloidin-AlexaFluor® 568 were obtained by confocal microscopy.

## CONCLUSION

- ✓ Ru agents **show high cytotoxicity and selectivity towards CRC cells, affecting the clonogenic ability** and **inducing apoptosis**.
- ✓ Ru agents **decrease motility at high concentrations** and **preferentially localize in membrane and cytoskeleton** in CRC cells.
- ✓ Ru agents also **affect F-actin polymerization** suggesting that actin might be a possible target for these compounds.


**References:** 1. Bray, F et al. A Cancer Journal for Clinicians, 2018. 68: 394-424. GLOBOCAN, 2018; 2. Hong, S et al. Cancer Medicine, 2016. 5: 248-255; 3. Bergamo, A et al. Journal of Inorganic Biochemistry, 2012. 106: 90-99; 4. Valente, A et al. Journal of Inorganic Biochemistry, 2013. 127: 79-81; 5. Garcia, M et al. WO 2016/087932.



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