The 8th International Electronic Conference on Medicinal Chemistry (ECMC 2022)
01–30 NOVEMBER 2022 | ONLINE

New ruthenium-cyclopentadienyl complex target specifically mutated KRAS in colorectal cancer

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Co-Chaired by PROF. DR. MARIA EMÍLIA SOUSA

pharmaceuticals MDPI

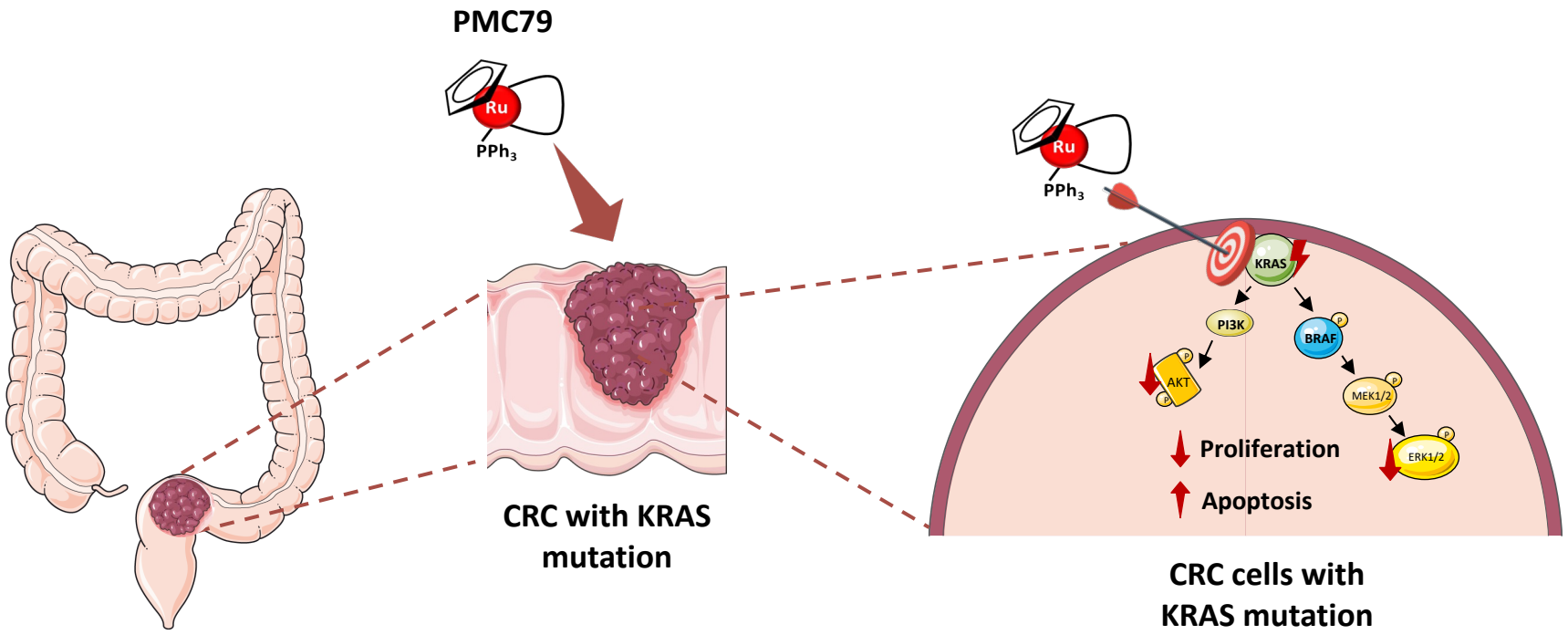
Ana Rita Brás[1,2,3*], Ana Lopes[3,4], Nuno Mendes[4,5], Fátima Gärtner[4,5,6], Ana Preto[1,2#], Andreia Valente[3#]

[1] Centre of Molecular and Environmental Biology, University of Minho, Braga, Portugal; [2] Institute of Science and Innovation for Bio-Sustainability, University of Minho, Braga, Portugal; [3] Centro de Química Estrutural, Faculty of Sciences of University of Lisbon, Lisbon, Portugal; [4] Institute for Research and Innovation in Health, University of Porto, Porto, Portugal; [5] Institute of Molecular Pathology and Immunology of the University of Porto, Porto, Portugal; [6] Institute of Biomedical Sciences of Abel Salazar, University of Porto, Porto, Portugal

#Co-senior authorship
* Corresponding author: pg31015@alunos.uminho.pt

New ruthenium-cyclopentadienyl complex target specifically mutated KRAS in colorectal cancer

Graphical Abstract



Abstract

Colorectal cancer (CRC) is an important cause of global morbidity and mortality. CRC harboring KRAS mutations accounts for 40% of all CRCs and is resistant to available EGFR inhibitors. Specific targeting of KRAS hotspot mutations is very difficult to achieve, highlighting the need of developing new specific target drugs. In this work, we aimed to evaluate the in vitro anticancer effects and explore the preclinical in vivo “proof of concept” for KRAS-mutated CRC therapy of a new family of ruthenium-cyclopentadienyl complexes.

CRC-derived cell lines with KRAS wild-type and different hotspot mutations were used to determine the phenotypic alterations induced by the complexes. A xenografted CRC mice model was used to determine in vivo toxicity and anti-tumor growth effect.

Our results revealed that Ru complexes are more cytotoxic for CRC cells, decreasing proliferation and inducing apoptosis. Studies with the PMC79 compound showed a decrease in the expression levels of KRAS, ERK and AKT proteins only in CRC-derived cells with KRAS mutation. In the in vivo therapeutical study, tumors treated with PMC79 had a much higher level of necrosis. Furthermore, the expression levels of KRAS, ERK and AKT proteins were also decreased in this model.

Overall, PMC79 has a noticeable effect inhibiting KRAS on CRC cells harboring KRAS mutation and not on CRC cells with KRAS wild type. This new Ru agent is a promising new drug for CRC therapy, suggesting to be a specific and a potential “magic bullet” for CRCs harboring mutated KRAS.

Keywords: Colorectal cancer, KRAS, ruthenium-cyclopentadienyl complexes

Colorectal cancer (CRC)

- 2nd leading cause of cancer death worldwide

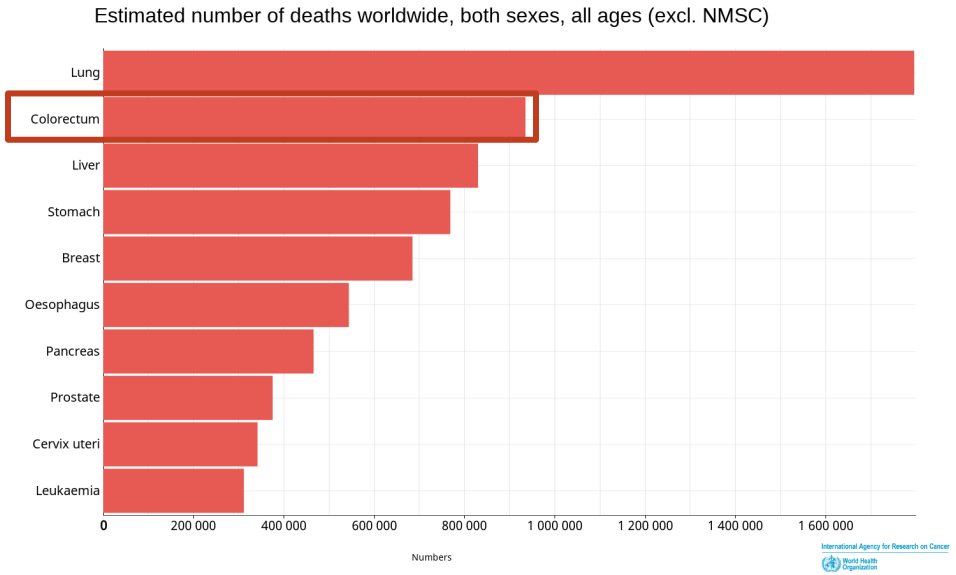


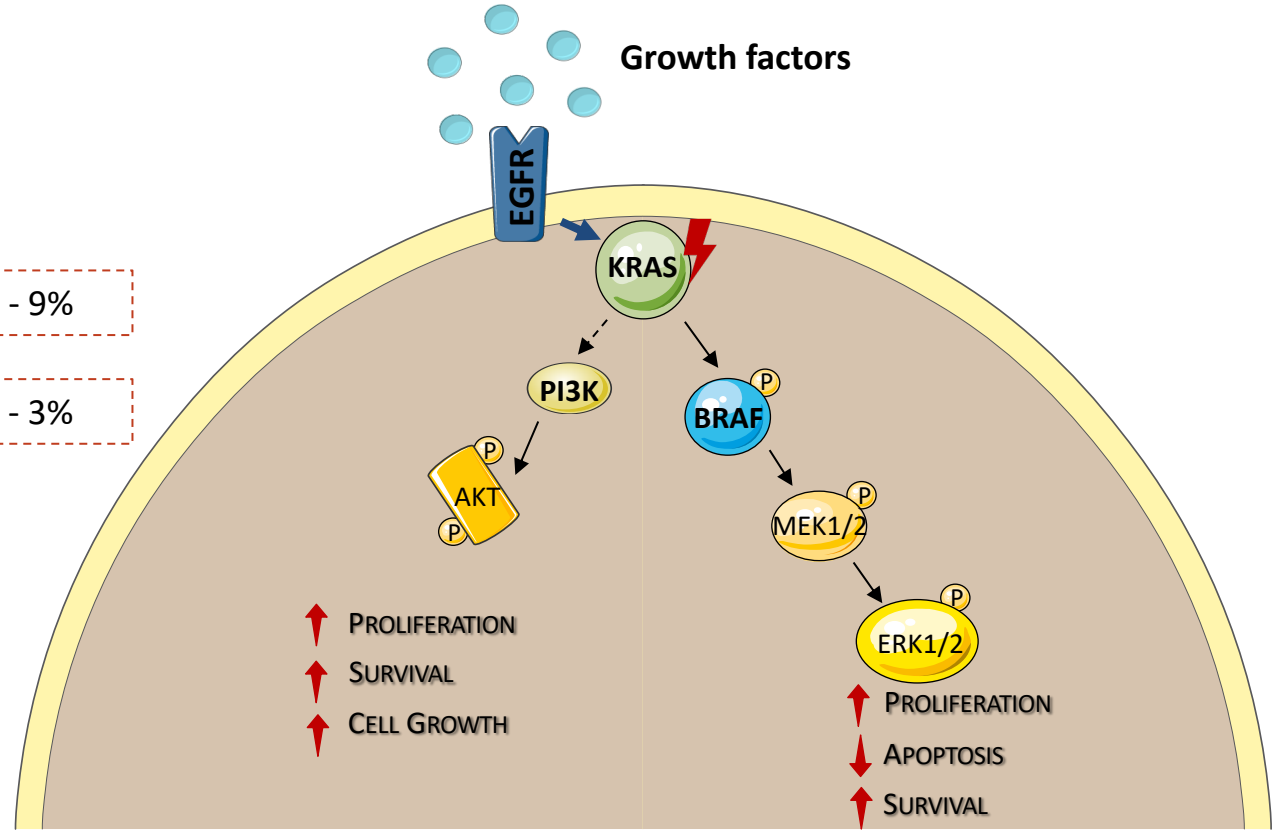
Figure 1. Number of estimated deaths worldwide in GLOBOCAN 2020. Data source: GLOBOCAN 2020. Global Cancer Observatory (http://gco.iarc.fr/).

KRAS mutations are high prevalent in CRC

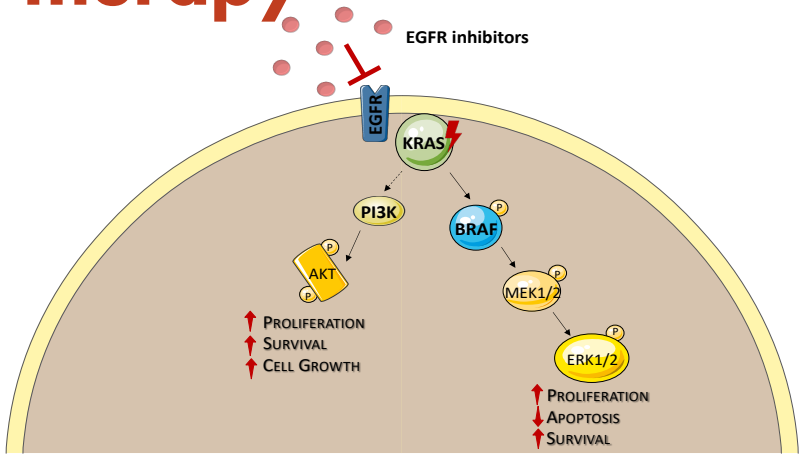
KRAS – 40%

Hotspot mutations:

- $KRAS^{G12D}$ - 13%
- $KRAS^{G12V}$ - 9%
- $KRAS^{G13D}$ - 7%
- $KRAS^{G12C}$ - 3%



Colorectal cancer therapy



Surgery

Chemotherapy

5-Fluorouracil

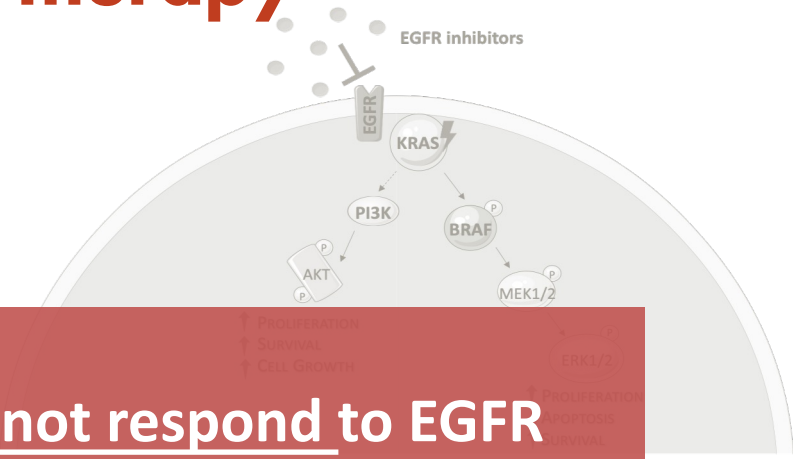
The most used chemotherapy agent to treat CRC

- **Success rate**: 10-15%
- Severe **side effects**
- **Resistance**

Targeted therapies

Specific targeting of proteins involved in growth and survival of cancer cells - **EGFR inhibitors**

Colorectal cancer therapy



Surgery

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The most used chemotherapy agent to treat CRC

- Success rate: 10-15%
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Targeted therapies

Specific targeting of proteins involved in growth and survival of cancer cells - EGFR inhibitors

CRC with KRAS mutations do not respond to EGFR inhibitors

Clinical relevant problem that needs to be overcome!

KRAS inhibitors

Two KRAS^{G12C} inhibitors: sotorasib and adagrasib, have been accepted for clinical use in non-small cell lung cancer (NSCLC)

To the best of our knowledge, there are no KRAS inhibitors available to specifically target KRAS hotspot mutations in CRC

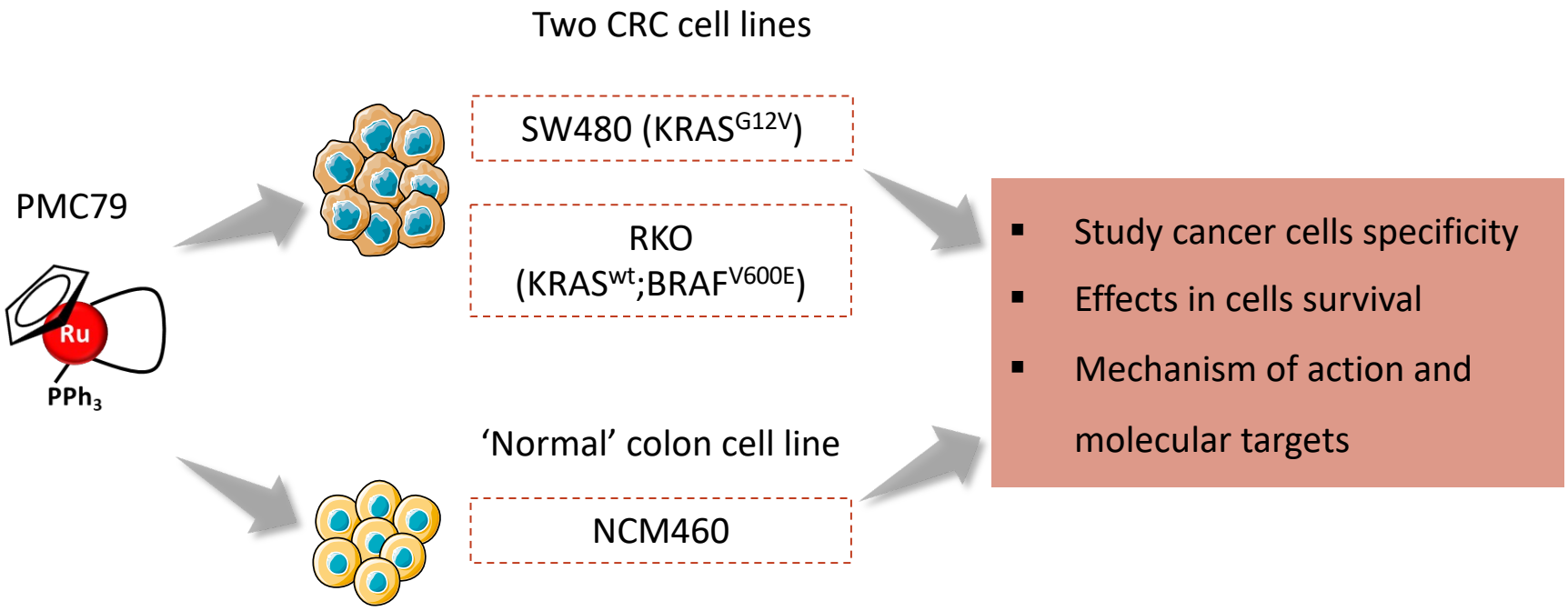
Ruthenium drugs: the next generation of metal-based compounds

- High activity – low doses to affect cancer cells
- Selectivity to cancer cells
- Structural diversity

Figure 2. Magic bullet. Adapted from: Valente & Zinck, 2015

Aim

Study the anticancer effect of PMC79 in colorectal cancer harbouring different mutations



PMC79 is selective to CRC cells

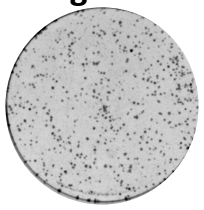
Table 1. IC_{50} values and selectivity index determined at 48 h of incubation with PMC79 and cisplatin by Sulforhodamine B.

	IC_{50} (µM)			Selectivity Index	
Compounds	**SW480^{KRAS}**	**RKO^{BRAF}**	**NCM460**	**NCM460/ SW480**	**NCM460/ RKO**
PMC79	40.0 ± 2.0	3.0 ± 0.5	44.0 ± 6.9	1.1	14.7
Cisplatin	7.0 ± 1.7	12.5 ± 2.7	6.3 ± 0.9	0.9	0.5

PMC79 decreases the clonogenic ability of CRC cells

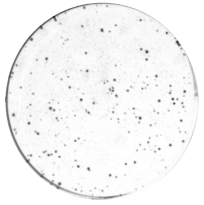
SW480^{KRAS}

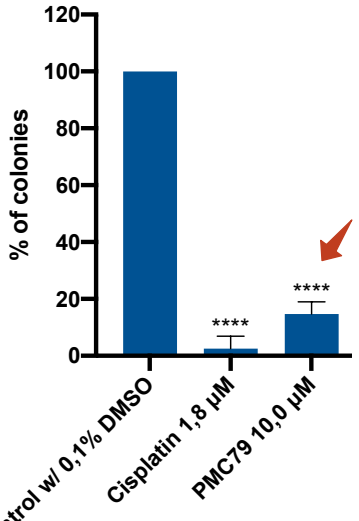
Neg. Control



Cisplatin

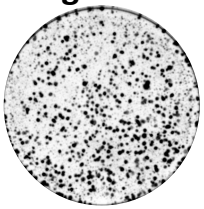
PMC79





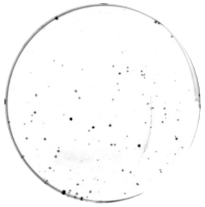
RKO^{BRAF}

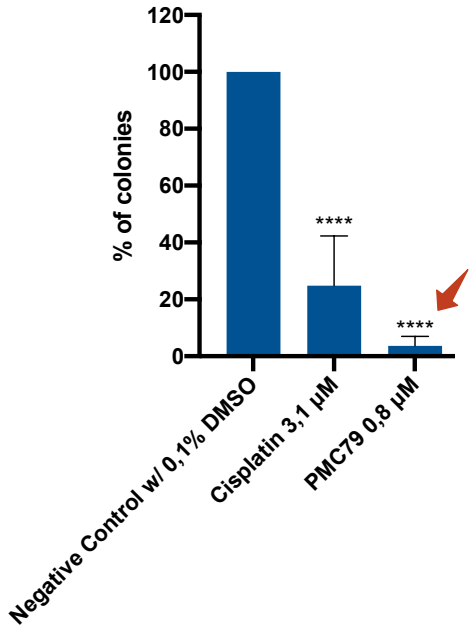
Neg. Control



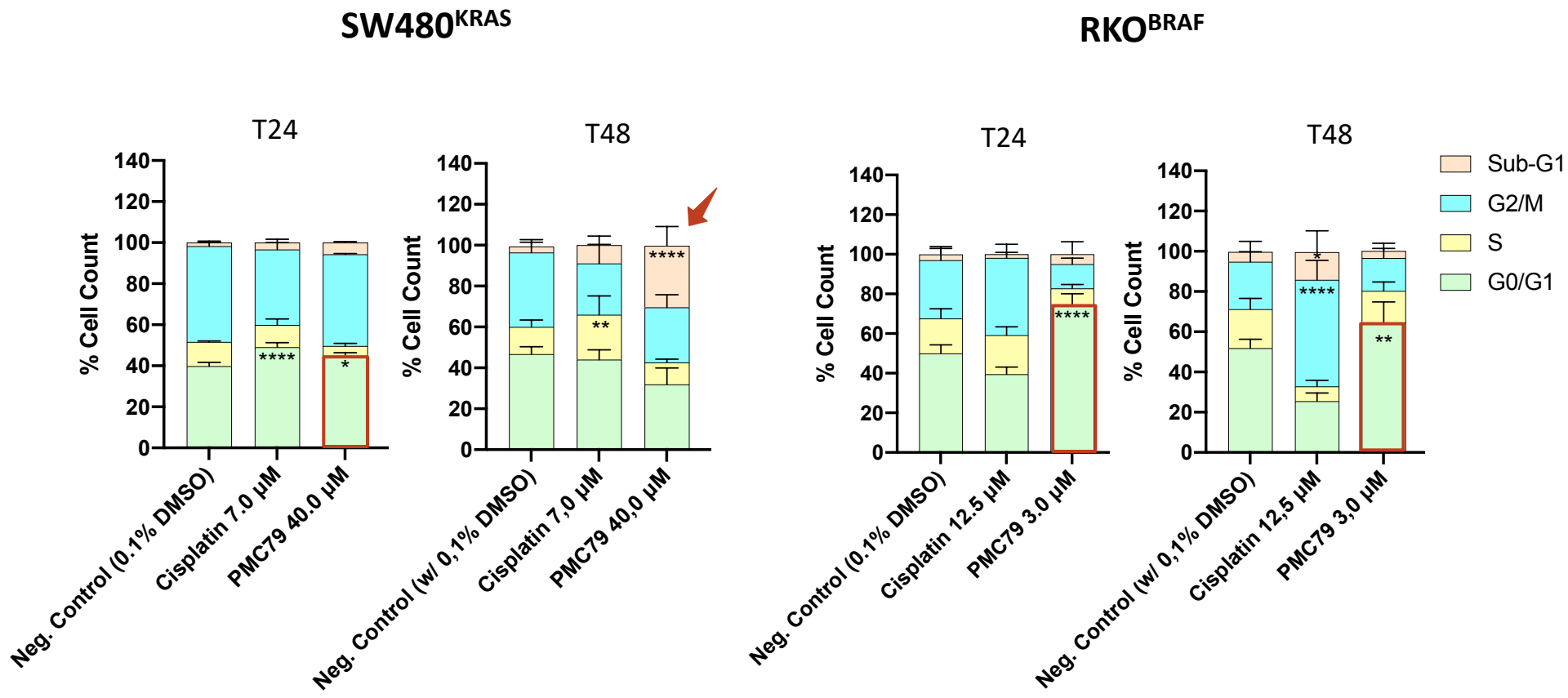
Cisplatin

PMC79

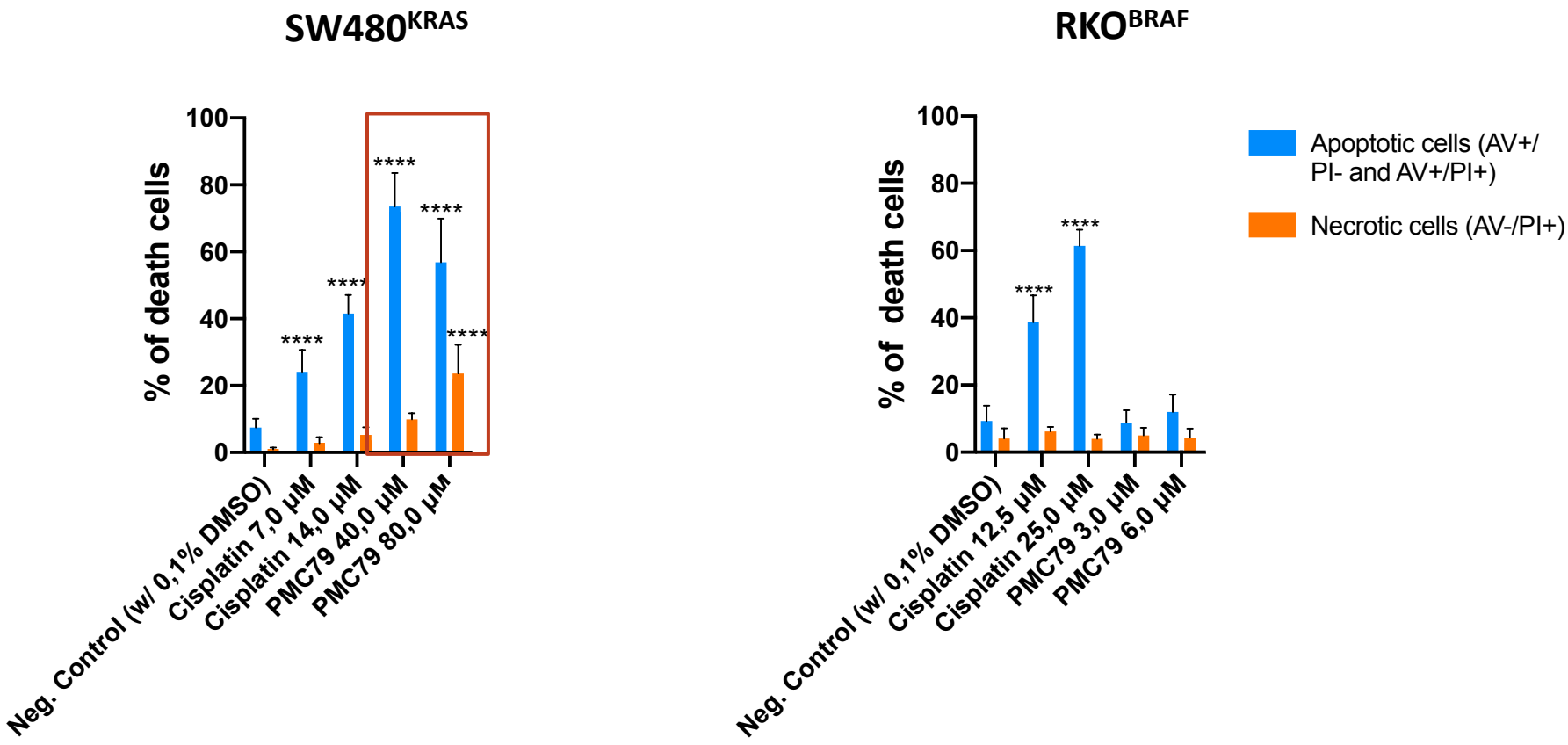




PMC79 induces cell cycle arrest in CRC cells



PMC79 induces apoptosis only in CRC cells with KRAS^{G12V}



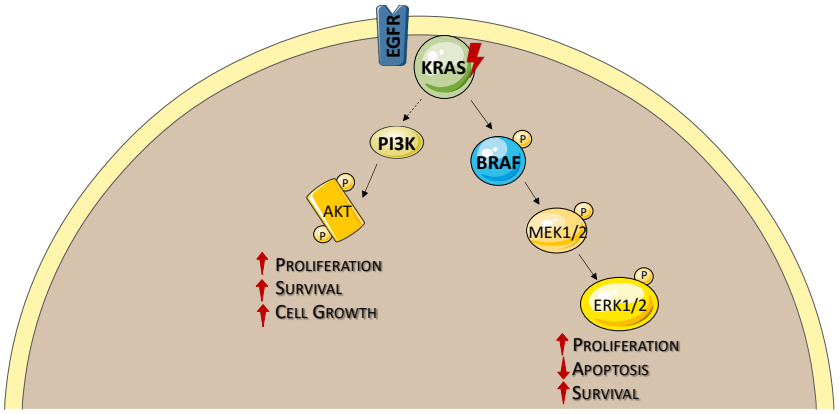
PMC79 inhibits KRAS and KRAS signaling molecules only in CRC with KRASG12V mutation

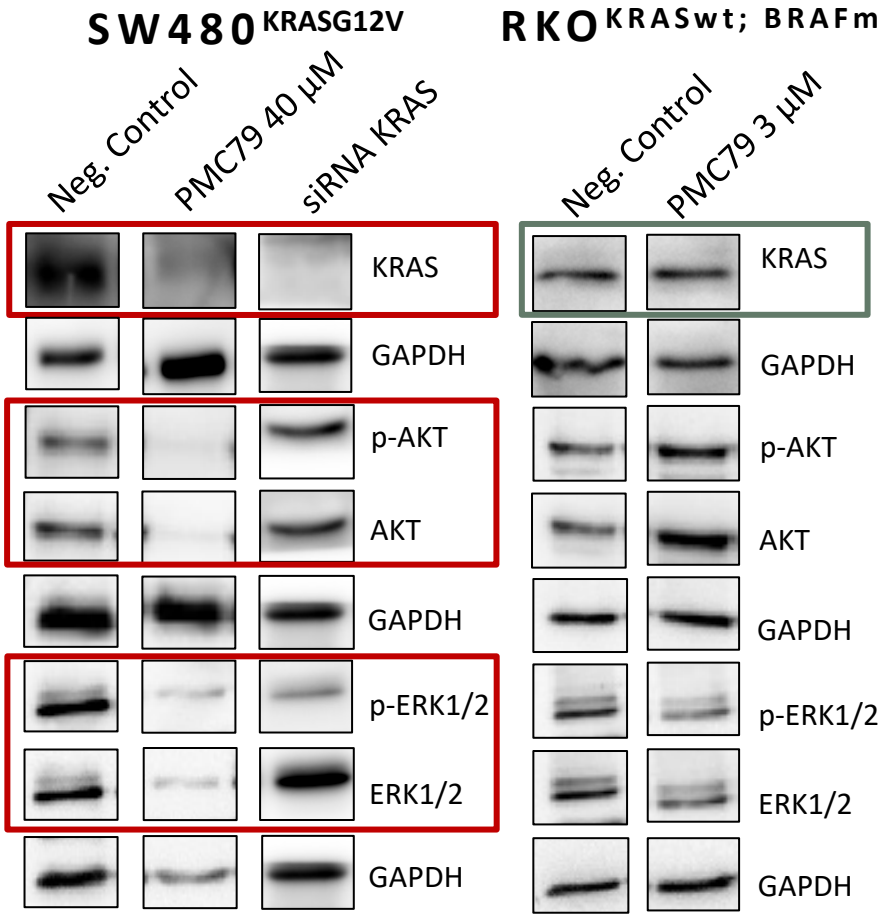
KRAS^{G12D} - 13%

KRAS^{G12V} - 9%

KRAS^{G13D} - 7%

KRAS^{G12C} - 3%





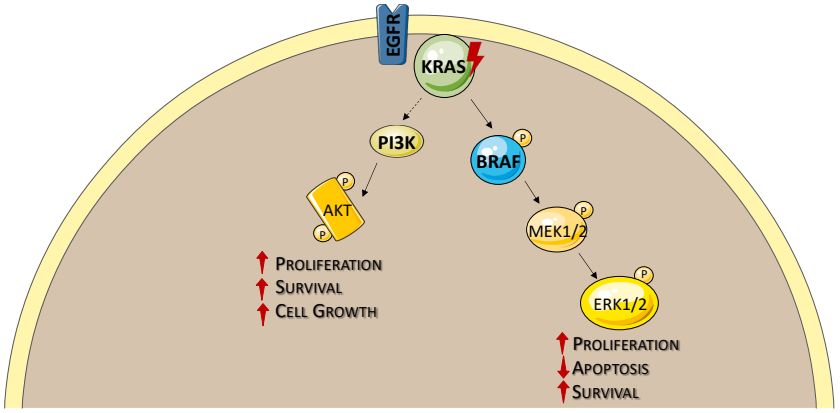
PMC79 inhibits KRAS and KRAS-signaling molecules in CRC with KRASG12V and KRASG12D mutations

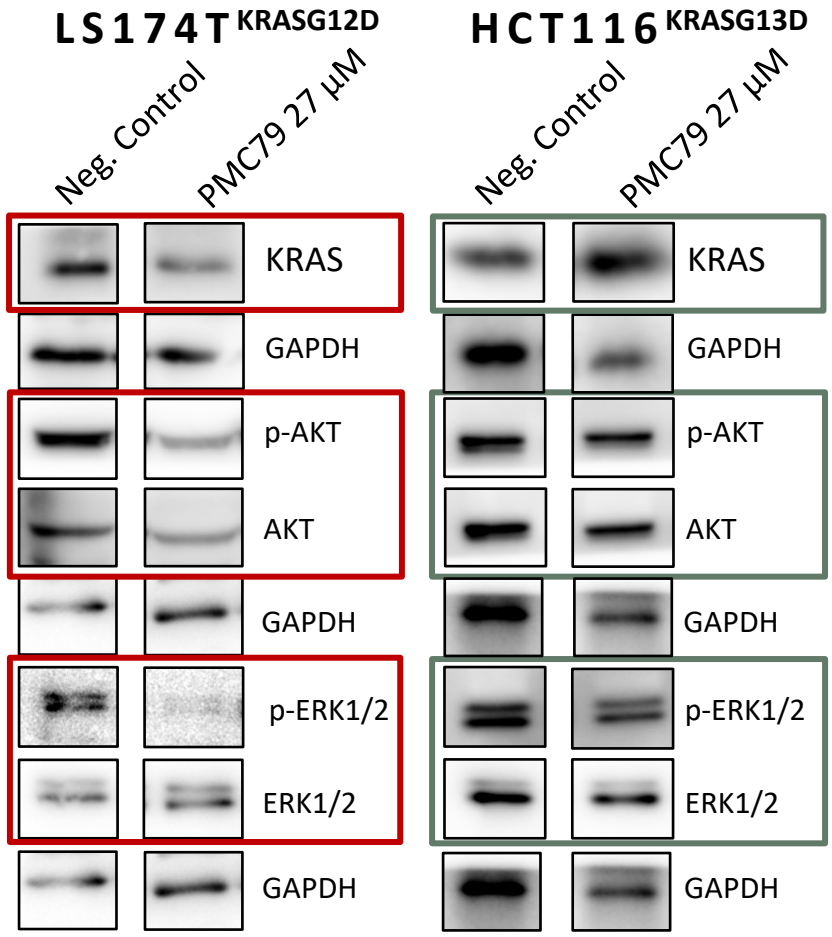
KRAS^{G12D} - 13%

KRAS^{G12V} - 9%

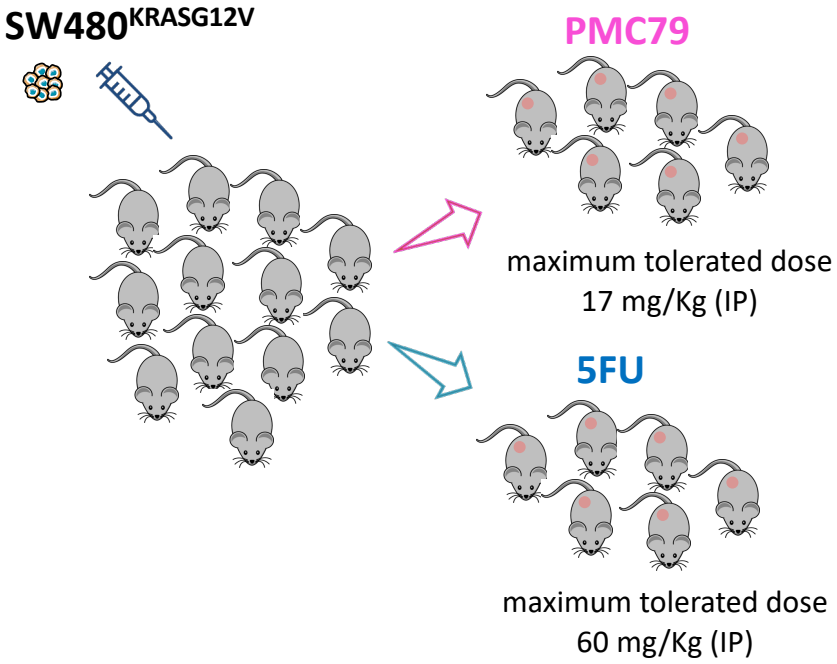
KRAS^{G13D} - 7%

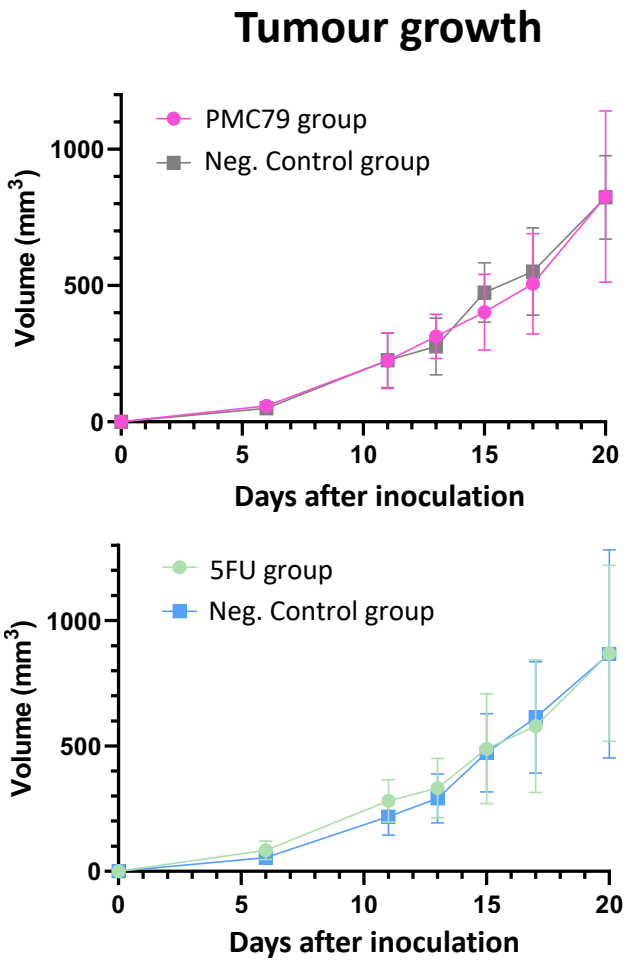
KRAS^{G12C} - 3%



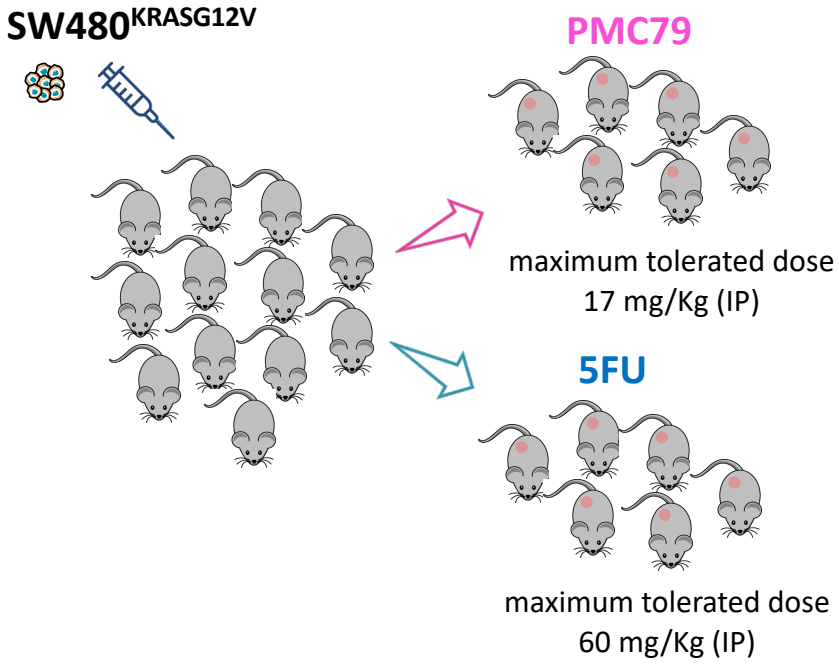


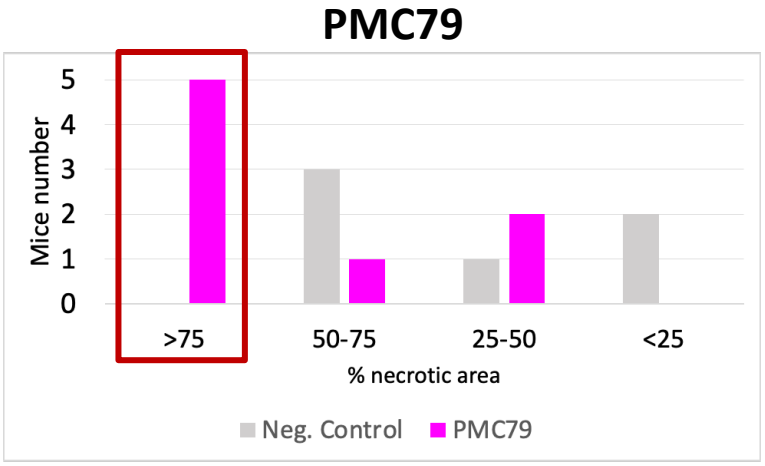
PMC79 have no effect in the tumour volume in xenografted CRC mice model

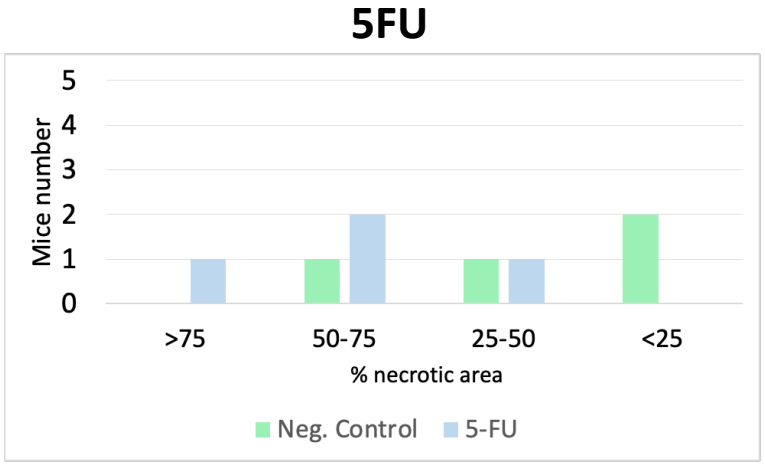




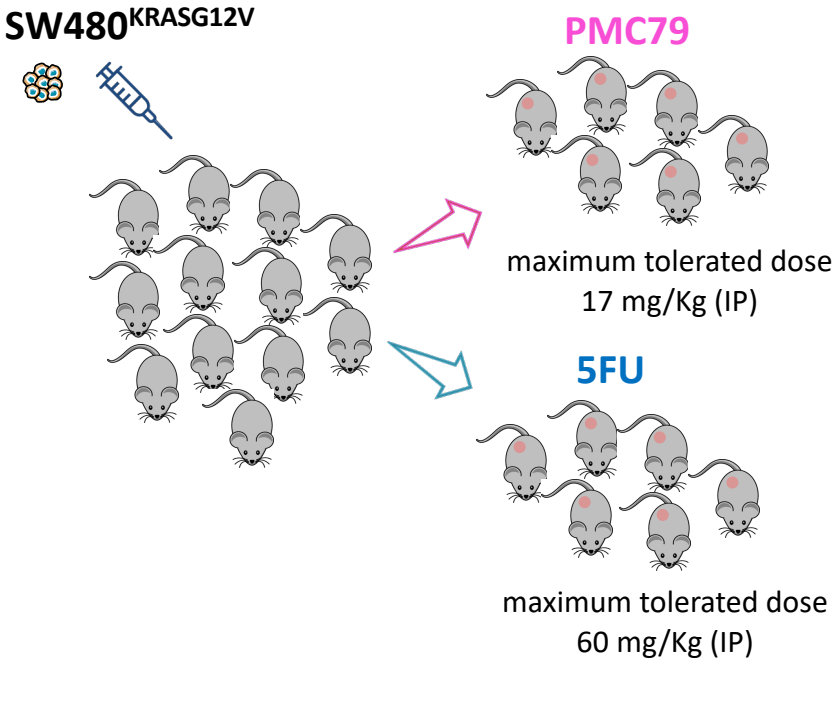
PMC79 increases the percentage of necrotic area in the tumours of xenografted CRC mice model

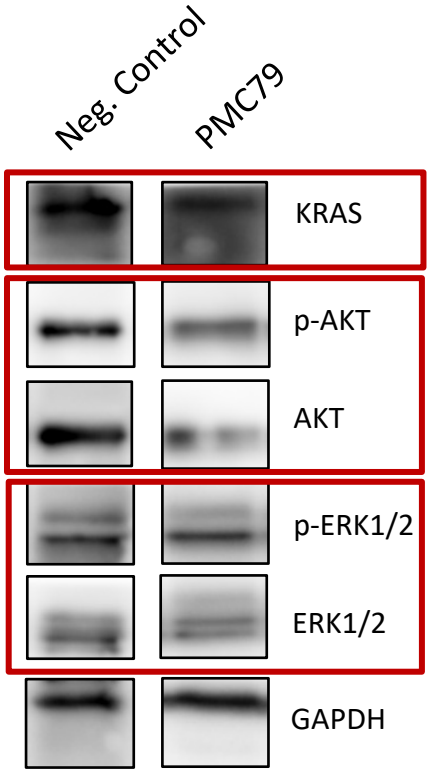


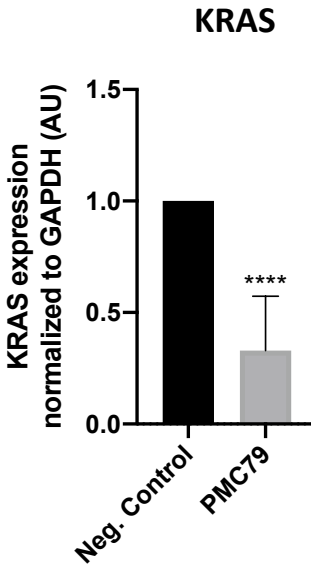




PMC79 decreases KRAS and KRAS-signaling molecules in the tumours of xenografted CRC mice model

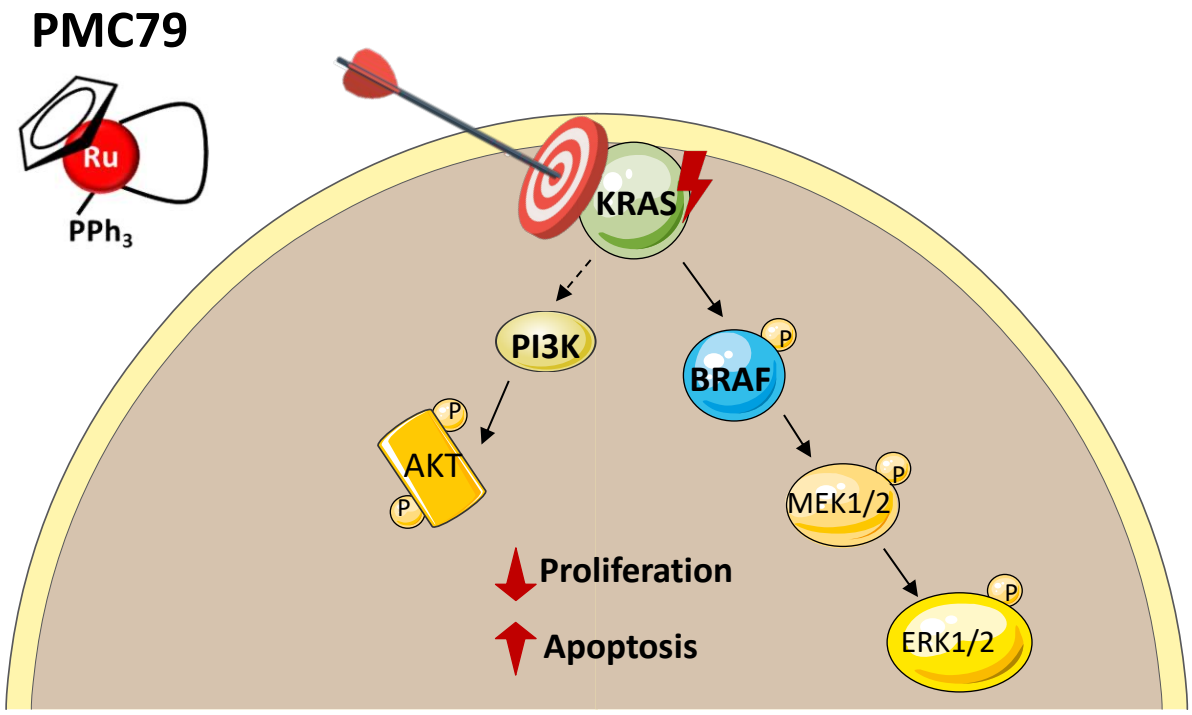






Conclusion

PMC79: NEW MAGIC BULLET TO TARGET KRAS G12V AND G12D MUTATIONS IN CRC



Patent submitted:

PPP 117807

Thank You!

Ana Preto's Group

Andreia Valente's Group

IBS centre of molecular and environmental biology

Ciências ULisboa

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FCT Fundação para a Ciência e a Tecnologia
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