

PTU-104

**ANTISENSE CHEMORADIOIMMUNOTHERAPY
CONSISTING OF ANTI-CASM SCFV LINKED ONTO
HIGH ENERGY RADIOISOTOPES, DOCETAXEL, AND 21
NUCLEOTIDE DOUBLE STRANDED siRNA TARGETED
TO DNMT1 INDUCE APOPTOSIS/PCD TYPE I IN
PANCREATIC AND PERIAMPULLARY CARCINOMA**

doi:10.1136/gut.2011.239301.232

J Giannios,^{1,*} S Delis,² V Barbunis,³ E Maragudakis⁴ ¹*Translational Cancer Medicine, Erasimio Oncology Hospital, Athens, Greece;* ²*GI Surgery, Saint Olga Hospital, Athens, Greece;* ³*Clinical Oncology, Ippokraton Hospital, Athens, Greece;* ⁴*Radiation Oncology, IASO Hospital, Athens, Greece*

Introduction Failure of tumour cells to undergo PCD cause resistance to chemoradiological therapies due to overexpression of oncogenes and transcriptionally repressed apoptotic TSGs due to methylation.

Methods We obtained surgically 19 pancreatic Ca specimens from 12 males and 7 females consisting of 9 ductal adeno Ca, 4 Ca of the papilla of Vateri, 1 acinus Ca, 1 neuroendocrine Ca, 1 intrapancreatic distal bile duct and 3 periampullary Ca. Genomic DNA of tumours was analysed for CpG island

hypermethylation by using MS-PCR. All of the tumours showed hypermethylation of TSGs: DUSP6/MKP-3 88%, RASSF1A 80%, RARb2 70%, BRCA1 50%. IHC, WB, SB and RT-PCR exhibited overexpression of DNMT1, CaSm, mcl-1 and bcl-2. We treated the samples with the isolated tumour cells with anti-CaSm scFv attached onto high energy-radioisotopes, docetaxel and 21-nucleotide double-stranded siRNA segment generated against DNMT1

Results Post-treatment, we detected re-expression of DUSP6/MKP-3, RASSF1A, RARb2, BRCA1 after inhibition of DNMT1 mRNA. There was downregulation of oncogene CaSm due to targeted scFv and inactivation of bcl-2 and mcl-1 due to phosphorylation by docetaxel. We detected upregulation of p21waf1, p27kip, Bid and Bak. The radioisotopes induced DNA double strand breaks in tumour cells arresting synergistically with docetaxel their growth at G2/M. We detected externalisation of PS, depolarisation of $\Delta\psi$ M, activation of caspases 3, 7, 8, 9, bax, cleavage of PARP and DNA fragmentation. TEM exhibited apoptotic bodies which were phagocytosed by adjacent tumour cells leading to a bystander killing effect.

Conclusion We have achieved to eradicate pancreatic Ca cells with combined chemoradioimmuno-therapy after circumvention of chemo- and radioresistant mechanisms such as hypermethylation of TSGs RASSF1A, RARb2, DUSP6/ MKP-3, BRCA1 and overexpression of oncogenes bcl-2, CaSm and mcl-1.

Competing interests None.

Keywords chemoradioimmunotherapy, scFv, siRNA, DNMT1, radioisotopes, pancreatic & periampullary Ca.