

**European Society of Medical Oncology (ESMO)  
Fellowships Program**

**ESMO Translational Research fellowship  
Progress Report**

**Research Fellow  
Andrea Alimonti, MD**

## **SUMMARY AND BACKGROUND**

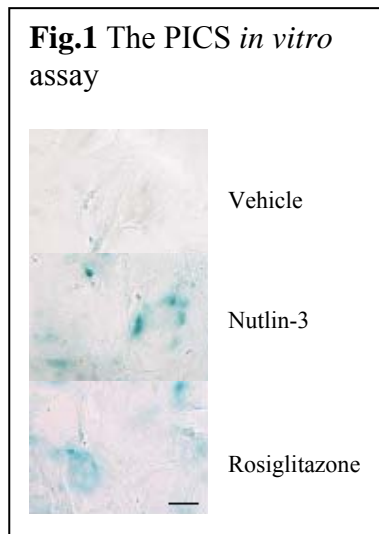
In the past years, my research has focused on the characterization of a novel type of cellular senescence response that we have identified in response to complete PTEN functional loss, and which we believe offers a radical therapeutic approach to target quiescent cancer stem cells, *in vivo*. As previously reported in my original application, in characterizing the mechanisms and features of *Pten* loss Induced Cellular Senescence, which we have named PICS for short, we have discovered that PICS is a distinct form of cellular senescence with several unique differences from oncogene-induced senescence (OIS) and replicative senescence (Di Micco, Nature 2007). These distinct differences include a lack of both DNA damage and hyper-replication; breaking the current dogma for senescence induction. In addition, we have discovered that PICS can be evoked even in non-proliferating cells. The ability to induce senescence in cells by targeting PTEN signaling, without a requirement for hyper-replication and DNA damage, opens up the possibility to target quiescent cells including quiescent cancer initiating cells (qCICs). Furthermore, the lack of a DNA damage response in PICS renders it possible to morph this senescence to an apoptotic response using DNA damaging agents and ionizing radiation. Since heterozygous PTEN mutation/deletion or protein down regulation is associated with a vast majority of prostate cancers (PCas) at presentation, this approach has tremendous therapeutic potential and represents one of the most exciting developments for the advancement of PCa prevention and therapy in recent years.

## **RESEARCH IN PROGRESS**

Thanks to the economical support granted by the ESMO fellowship in translational research, we have achieved impressive results in the field of “pro-senescence” therapy for cancer. Our major advances have been on the *in vivo* characterization of enhancers of PICS in combination with the mTOR inhibitor RAD001 and on the identification of novel “pro-senescence” compounds. Below we include to the attention of the ESMO fellowship committee, a detailed research report of all the results achieved for each proposed aims.

**Specific Aim 1: A small molecule screening to identify enhancers of *Pten*-loss induced cellular senescence**

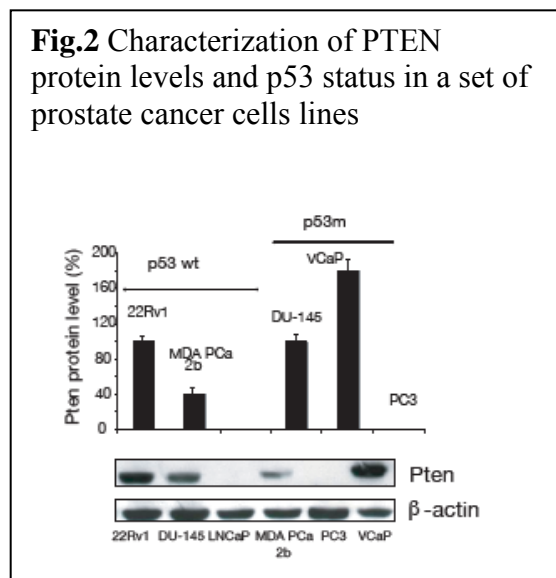
One of the main objectives of this fellowship is the identification of compounds with the potential to increase *Pten*-loss induced cellular senescence. As previously reported in our original application, we have recently patented an *in vitro* senescence assay (PICS assay) that can allow us to quickly identify enhancers of PICS (Int. App. No.:PCT/US2008/07897). To avoid time



consuming and expensive compounds screening we have decided to assess in our assay, the pro-senescence efficacy of previously patented compounds of known and unknown anti-cancer potential in (Fig.1). This strategy is allowing us to cut the costs linked to the development of identified leads and to move directly to their pre-clinical phase. Up today, we have identified five enhancers of PICS. Between these five leads, Rosiglitazone a PPARgamma agonist (Ray DM, J Immunol. 2006) shows the highest “pro-senescence” and growth inhibitor potential (Fig.1). As Nutlin-3, Rosiglitazone can increase the level of p53 in PICS cells driving a strong cellular senescence response. We have recently confirmed Rosiglitazone

“pro-senescence” activity also in a panel of human cancer prostate cell lines.

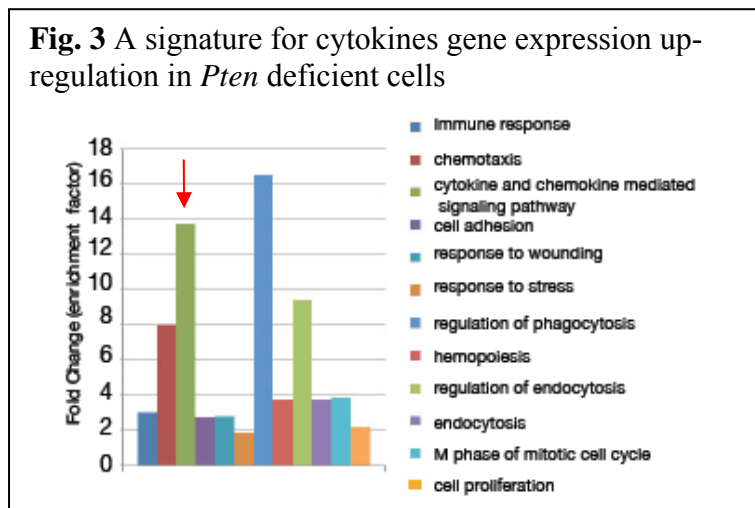
The characterization of this panel of prostate cancer cell lines on the basis of PTEN and p53 status (Fig.2) has also allowed us to validate in another setting the “pro-senescence” potential of previously identified compounds such as the PTEN inhibitor VO-OHPic (Rosivatz ACS Chem Biol 2006). In spite of the fact that these cell lines are derived from metastatic and advanced cancers, three were found to be wt for p53 with one cell line, MDA PC2b (Fig. 2), expressing at the same time reduced PTEN protein levels. On the basis of this stratification, we have studied the sensitivity of this cell line to a PTEN inhibitor. Remarkably, treatment with VO-OHPic resulted in a substantial increased in  $\beta$ -Galactosidase staining and a decreased Ki-67 staining when compared to the untreated cells.



In sum, these findings demonstrate that treatment with a PTEN inhibitor drives a strong senescence response. As PTEN inhibitors are already being proposed as potential drugs for treatment of other diseases, it is provocative to think that they may also be useful in the prevention and treatment of certain cancers. Finally, we have collected evidences that combination of the PTEN inhibitor VO-OHpic with the MDM-2 inhibitor Nutlin-3 can convert PICS into an apoptotic response in MDA prostate cancer cell line (Fig.2). This suggests that in certain conditions senescence can be morphed into an apoptotic response definitely removing the pool of arrested senescence cells from the tumor. **Future directions:** Complete the screening of compounds with “pro-senescence” activity in the PICS assay, test novel combination of pro-senescence compounds *in vitro*. Start a pre-clinical trial to assess the efficacy of PPARgamma agonists in *Pten* null prostate conditional mice

***Specific Aim 2: To assess whether PICS acts together with the innate immune system to limit tumor growth***

Another main objective of this proposal is to evaluate whether *Pten* complete loss or *Pten* pharmacological inhibition, can drive the up-regulation of the gene expression of cytokines which can potentially trigger a peri-tumoral immune-response. Previous papers have demonstrated that cellular senescence, driven by p53 reactivation, can trigger the up-



regulation of chemokines and/or adhesion molecules that have the ability to recruit peri-tumoral polymorphonuclear leukocytes and promote tumor clearance (Lowe S, Nature 2007). In these last months we have carried on sets of experiment on the gene expression profile of *Pten* deficient cells and we have discovered that in these cells there is a strong and statistically significant up-regulation of several genes encoding for cytokines (see Fig. 3, red arrow). These findings confirm previous observations from the group of S. Lowe at Cold Spring Harbor and open up the possibility that therapies which increase senescence by inhibiting PTEN or MDM2 may promote a tumor immune response resulting in tumor clearance. Thus we are very exciting to validate *in vivo*, whiter the anti-tumor effect of compounds such as IL2 can be potentiated by compounds that inhibit PTEN or

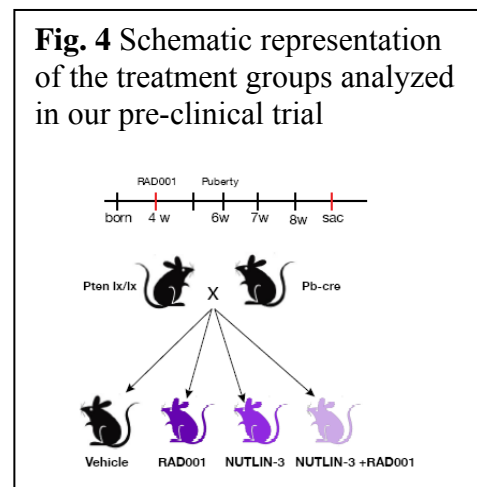
increase senescence by inhibiting MDM2, such as Nutlin-3 (see Specific Aim 3). This part of the project will be central in the second year of my fellowship.

**Future directions:** Validation of the identified genetic signature (see Fig.3) by RT-PCR in *Pten* null MEF and human prostate cancer cell lines.

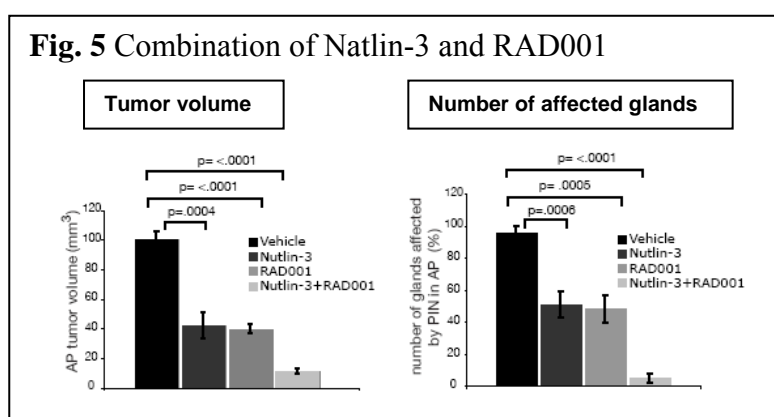
Start a pre-clinical trial to study the efficacy of the combination of IL2 and Nutlin-3 in *Pten*<sup>PC-/-</sup> mice.

**Specific Aim 3: To evaluate the efficacy of “pro-senescence” therapy in preclinical mouse models.**

As previously mentioned in the introduction of our progress report, during these months we have focused in the validation of the *in vivo* efficacy of compounds with pro-senescence potential in our *Pten* null prostate conditional mouse model (*Pten*<sup>PC-/-</sup> mice). We are very exciting to have almost concluded a pre-clinical trial assessing the efficacy of Nutlin-3 a novel inhibitor of MDM2 (Vassilev LT. Science 2004) alone and in combination with the mTOR inhibitor, RAD001 (see Fig.4, for a schematic representation of the treatment groups).



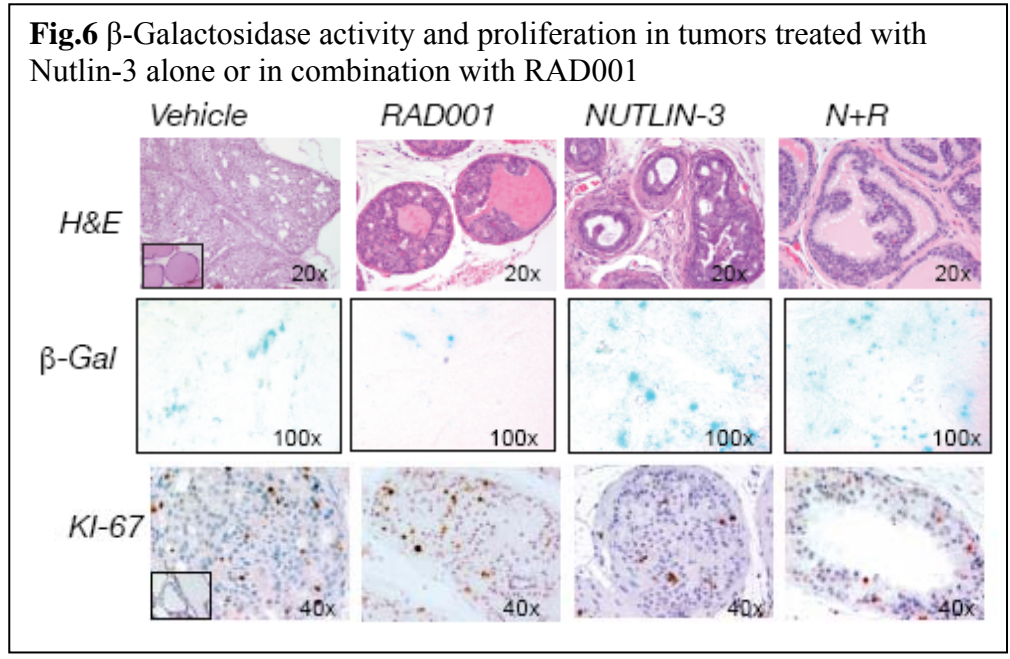
Our new findings demonstrate that Nutlin-3 alone can decrease both the tumor size and the number of prostate affected glands (Fig.5). This is associated with an increase in three well characterized senescence markers,  $\beta$ -Galactosidase activity, p53 and p21 staining in all the prostate



glands analyzed (Fig. 6). By contrast, although growth inhibitory, RAD001 markedly decreased senescence in almost all the remaining-affected glands of the treated mice as visualized by both a decrease in  $\beta$ -Galactosidase activity, p53 and p21 staining (Fig. 6). Combination of

both Nutlin-3 and RAD001, rescued the senescence phenotype, as demonstrated by the increased tumors  $\beta$ -Galactosidase activity, p53 and p21 staining in mice treated with the combination of the two drugs when compared with the mice treated with RAD001 alone (Fig. 6). The rescue of senescence resulted in a dramatic reduction of tumor size, number of affected glands (Fig. 5) and percent of proliferating tumor cells (Fig. 6). Notably, staining by TUNEL revealed no difference in

apoptosis between the treatment groups, suggesting that the main function of Nutlin-3 in PICS is to enhance senescence rather than induce an apoptotic response. These findings validate the “pro-senescence” potential of Nutlin-3 in a genetically engineered mouse prostate model and represent a proof of principle example for the importance of the preservation of an intact senescence response



in cancer chemoprevention and therapy. Our findings also suggest that treatment with mTOR inhibitors should be combined with drugs that sustain the p53 response. This is particularly relevant

in prostate cancer, where p53 function often remains intact.

**Future directions:** *In vivo* characterization of the inflammatory response and cytokines up-regulation in the *Pten*<sup>pc-/-</sup> mice before and after treatment with Nutlin-3 and RAD001. Combination of Nutlin-3 (single administration) with irradiation in *Pten*<sup>pc-/-</sup> mice.