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MEETING ABSTRACTS

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Immunotherapy Bridge 2020 and Melanoma Bridge 2020: meeting abstracts

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Oral Communications

1.

Cytokines and immune cell populations performance during nivolumab treatment. A subgroup analysis of NIVACTOR study

Andrea Abbona[1]*, M. Paccagnella[1], Nerina Denaro[2], A. Falletta[1], Danilo Galizia[3], Lorena De Zarlo[3], Erika Fiorino[5], Loretta Gammaitoni[4], Dario Sangiolo[5], Marco Carlo Merlano[1]
[1]Dipartimento di Oncologia, Azienda Ospedaliera St Croce e Carle, Cuneo, Italy; [2]Medical Oncology, Azienda Ospedaliera St Croce e Carle, Cuneo, Italy; [3]Medical Oncology, IRCCS Candiolo Institute, Turin, Italy; [4]Medical Oncology, FPO-IRCCS Candiolo Institute, Turin, Italy; [5]Department of Oncology, University of Turin, Turin, Italy
Correspondence: Andrea Abbona - abbona.andrea@gmail.com
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Background: Recurrent or Metastatic Platinum-refractory Squamous Cell Carcinoma of the Head and Neck (R-M SCCHN) is a major clinical issue with 1 year survival rate of 20–30% and a median overall survival (OS) of 10 months [1, 2]. Nivolumab, anti-PD1 mAb, is approved for 2nd line R-M HNC, but only 15–20% of patients will benefit [3]. Therefore, there is an unmet need for robust predictive markers for patient selection.
Patients and methods: We analyzed changes of plasma circulating cytokines and immune cells during treatment at baseline (T0), at day 1 cycle 3 (T1), at day 1 cycle 7 (T2) and at disease progression (TPD). Cytokines' concentrations were assessed using Simple Plexsystem (ProteinSimple). Immune cells from peripheral blood were analyzed by flow cytometry using FACS Cyan (Cyan ADP, Beckman Coulter) and analyzed with Summit Software. Principal component analysis (PCA) was performed to group patients with good or poor progression free survival (PFS) and OS using cut-off based on regression points of their extracted factors: group B (patients with factor 1 < 0.7 and factor 2 < 0.0) and remaining patients in group A.
P < 0.05 was considered to indicate significance.
Results: 18 patients were analyzed for both cytokine and immune cell populations. CCL-4 increased from T0 to T2 (P = 0.047) [Fig. 1]. Using PCA analysis we clustered patients in group B and group A on their T0 levels of IL-5, IL-6, $CD3^+CD8^+LAG3^+$, $CD3^+CD8^+PD1^+LAG3^+$ and $CD8^+$TEMRA [Fig. 2]. Then we performed Cox analysis using cut-off from regression points of their factors extracted with PCA observing a significant better PFS and OS in patients of group B (HR 0.185, P = 0.030 and HR 0.063, P = 0.010 respectively) [Fig. 3]. We also found that patients with increased levels of T_{reg} cells from T0 to T1 had better PFS (HR 0.098, P = 0.001) [Fig. 4]. Longitudinal analysis showed that TNF-α and IFN-γ increased from T0 to TPD (P = 0.049 and P = 0.035, respectively).

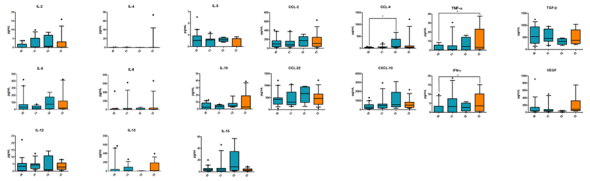

Fig. 1 (Abstract 1) Distributions of 17 plasma cytokine levels during nivolumab treatment. Cytokines concentration was expressed in pg/mL. Data are shown as median with range

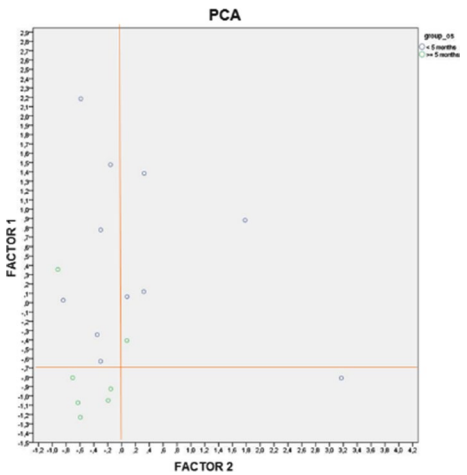

Fig. 2 (Abstract 1) PCA analysis using T0 values of IL-5, IL-6, $CD3^+CD8^+LAG3^+$, $CD3^+CD8^+PD1^+LAG3^+$ and $CD8^+$TEMRA as parameters. Group B includes patients (n = 5) with factor 1 < 0.7 and factor 2 < 0; group A includes the other patients (n = 13)

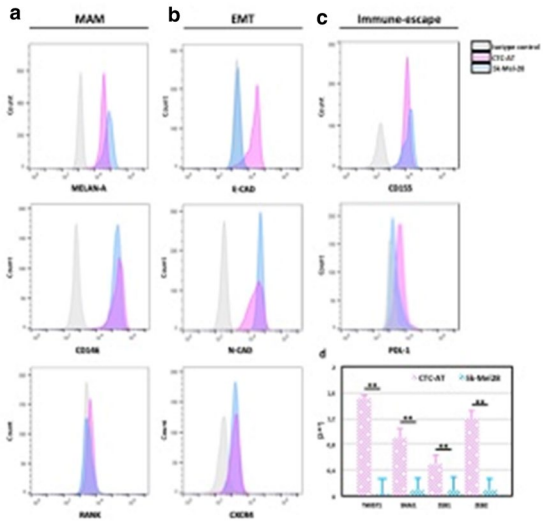

Fig. 1 (Abstract 13) The CTC-AT cell line shows typical melanoma phenotype (**a**), an intermediate EMT phenotype (**b-d**) and molecules associated with immune escape (**c**)

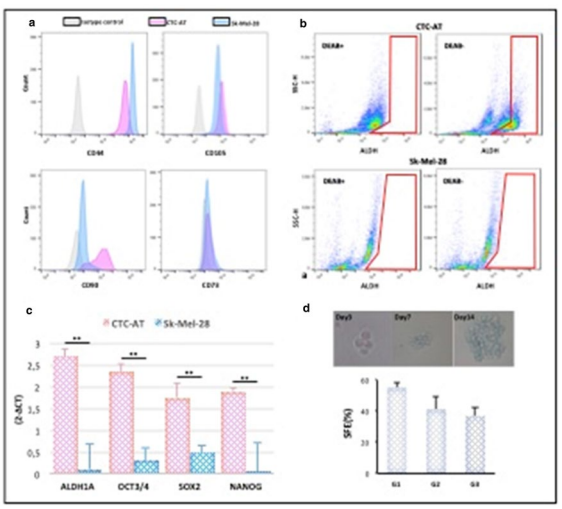

Fig. 2 (Abstract 13) The CTC-AT cell line shows molecular and functional features of cancer stem cells (CSC)

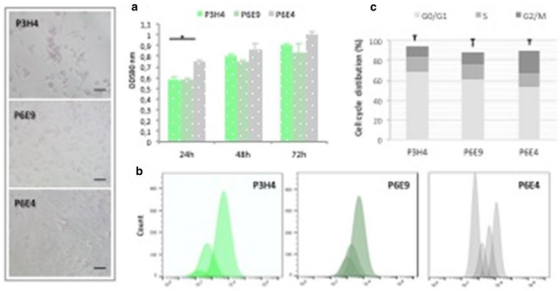

Fig. 3 (Abstract 13) The three clonal subpopulations are characterized by different proliferation rate

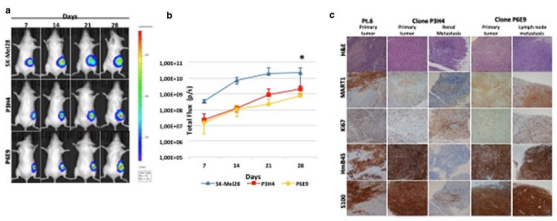

Fig. 4 (Abstract 13) The in vivo bioluminescence images display the tumorigenic potential of each clone (**a and b**) and the expression of typical melanoma markers by CTC-AT induced metastasis with respect to the primary tissue (**c**)

13.

The clonal heterogenicity of circulating tumor cells (CTCs) drives their metastatic potential: a new NOD-SCID melanoma model

Claudia Felici[1], Paola Cafforio[1], Francesco Mannavola[1], Stucci Luigia Stefania[1], Camillo Porta[1], Marco Tucci*[1]
[1]Department of Biomedical Sciences and Human Oncology, University of Bari Aldo Moro, Bari, Italy
Correspondence: Marco Tucci - marco.tucci@uniba.it
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Background: Innovative therapies have improved the overall survival in melanoma although a high number of patients still experience disease progression or recurrence. The clonal heterogenicity of melanoma cells is a critical issue concurring to drug-resistance development and metastatic spreading that, however, could be efficiently forecasted by CTC measurement. A number of studies attempted to validate a method for capturing viable CTCs in metastatic melanoma and a peculiar phenotype and biological properties have been recently correlated with outcome.
Materials and methods: CTCs were obtained from 15 patients with BRAFV600-mutated metastatic melanoma by DEPArray technology and cultured in vitro. Cells established in vitro were definitely transduced with luciferase. The mutational state was assessed by NGS. The CTC phenotype and molecular profile were investigated by cytometry and qPCR. Stemness was investigated by measuring sphere formation and ALDH activity, while limit dilution discriminated the existence of clonal subpopulations. Viability, proliferation and cell cycle were investigated. The tumorigenic potential of subclones was evaluated by their injection in 8-weeks old NOD/SCID mice. Mice were sacrificed while site of metastasis and tumor burden were evaluated by Lumina-SIII. Metastatic tissues were analysed by immunohistochemistry.
Results: A single cell line was established in vitro from a patient with the highest number (n = 102) of CTCs (CTC-AT). Missense mutations

of BRAF, TP53 and PIK3CA occurred in CTC-AT and primary tissue. An intermediate EMT phenotype was demonstrated as result of the SNAI1, TWIST1, ZEB1 and ZEB2 or both PDL-1 and CD155 levels (Fig. 1). The expression of CD44, CD90, CD10, CD73 or Oct3/4, Nanog and Sox2 confirmed the presence of stem cells, whereas the ALDH activity and sphere formation suggested the stemness (Fig. 2). Three clonal subpopulations showing different proliferative rate were obtained (Fig. 3). In vivo studies revealed the ability of two clones to develop the tumor, although they showed different metastatic potential (Fig. 4).

Conclusions: Herein we described a new NOD-SCID model of metastatic melanoma induced by CTCs. The heterogeneity of subclones as well as their stemness support the role of CTCs for investigating the variability of melanoma behaviour and offers the opportunity of pharmacogenomic studies to guide future therapeutic strategies in advanced disease.

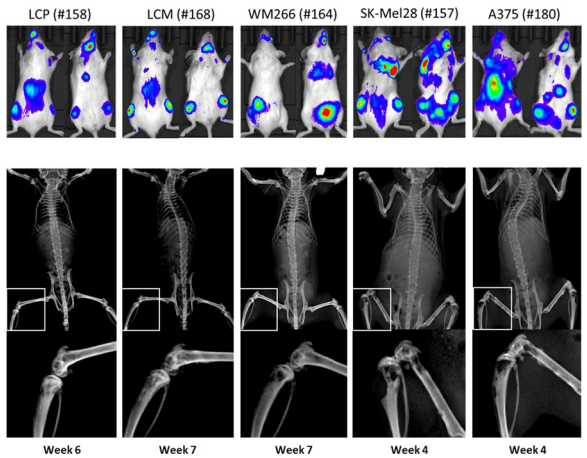

Fig. 1 (Abstract 14) Metastatic activity at different time points from intracardiac injection of five melanoma cell lines by in vivo bioluminescence *(upper panel)* and x-ray imaging *(lower panel)*

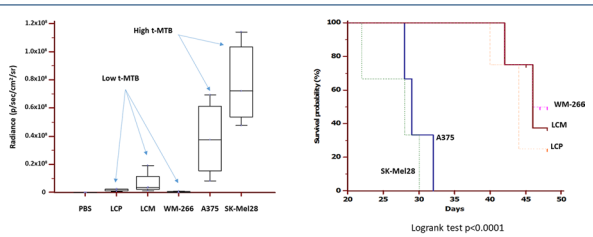

Fig. 2 (Abstract 14) Metastatic activity *(left)* and impact on mice survival *(right)* measured by total metastatic tumor burden (t-MTB) and Kaplan-Meyer's curves

14.

Characterization of the metastatic behaviour and gene expression profile (RNAseq) of different melanoma cell lines: a comprehensive in vivo model.

Francesco Mannavola[1], Domenica Lovero[1], Claudia Felici[1], Gerardo Cazzato[2], Marco Moschetta[3], Mauro Mastropasqua[2], Camillo Porta[1] and Marco Tucci*[1]

[1]Section of Medical Oncology, Department of Biomedical Sciences and Clinical Oncology (DIMO), University of Bari 'Aldo Moro', Bari, Italy; [2]Section of Pathology, Department of Emergency and Organ Transplantation (DETO), University of Bari 'Aldo Moro', Bari, Italy; [3]Breast Care Unit, Department of Emergency and Organ Transplantation (DETO), University of Bari Medical School, Bari, Italy

Correspondence: Marco Tucci - marco.tucci@uniba.it

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Background: Metastasis is the major cause of death in malignant melanoma. Several factors, including clinical-pathological and tumor biological features may restrain prognosis. Molecular mechanisms regulating melanoma progression and metastasis have been partially discovered, and thus we developed an in vivo model of metastatic melanoma to investigate potential genes implicated in these events.

Materials and methods: To evaluate the in vivo metastatic activity of five different melanoma cell lines (LCP, LCM, WM266, SK-Mel28 and A375), we completed intra-cardiac (ic) injection of 1×10^6 luminescent

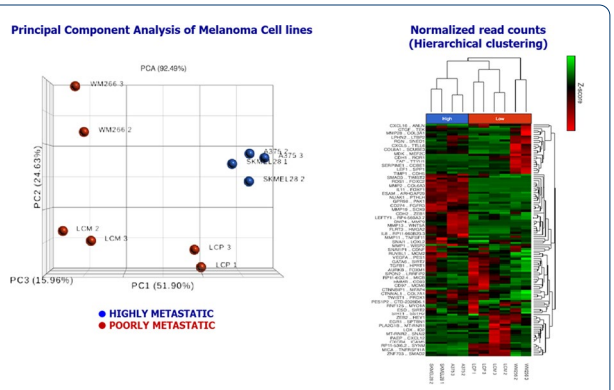

Fig. 3 (Abstract 14) Three-dimensional PCA plot (*left*) and 118 genes heatmap with "unsupervised hierarchical clustering" (*right*) by RNAseq of melanoma cell line

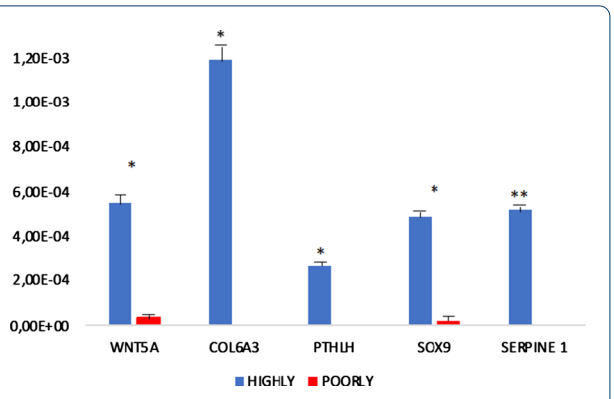

Fig. 4 (Abstract 14) Gene expression analysis on FFPE metastatic samples from highly (Group A) *vs* poorly (Group B) metastatic cell lines