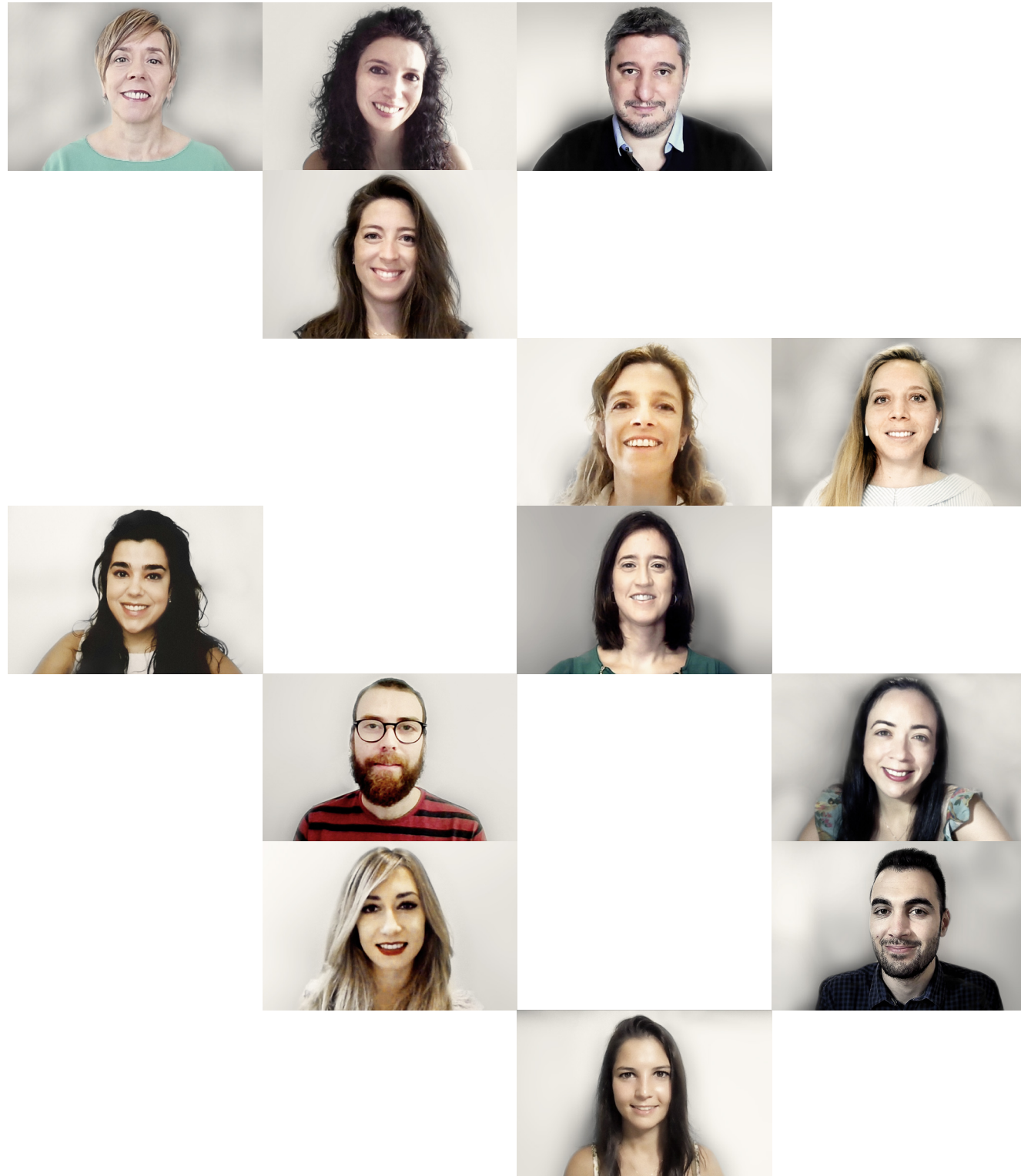


MELANOMA GROUP

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OVERVIEW

Melanomas are the only tumours where lesions barely over one millimetre in depth can be at risk for metastasis. An increasing number of (epi)genetic alterations and mechanisms of immune evasion have been identified in this disease. Nevertheless, no molecular biomarker has been approved as a *bona fide* prognostic indicator. The field is also in need of improved treatments, as a significant fraction of patients is resistant to targeted and immune-based therapies. The long-term goal of our Group is to identify new progression biomarkers and anticancer agents. We are particularly interested in defining lineage-specific vulnerabilities that distinguish melanomas from other tumours with lower metastatic potential (publications in *Nature*, *Cancer Cell*, *Nature Cell Biology*, *Nature Communications*, among others). Our laboratory has also generated first-in-class lymphoreporter mice for non-invasive imaging of pre-metastatic niches in melanoma (*Nature*) and has identified actionable immune suppressive mechanisms with implications for patient treatment (*Nature Medicine*). Our ultimate objective is to improve the management of patients with otherwise refractory metastatic melanomas.

“We have visualised and targeted (pre)metastatic niches in melanoma and defined mechanisms of immune suppression with clinical implications for cancer patients.”

RESEARCH HIGHLIGHTS

CNIO Melanoma Group: objectives and model systems

Melanomas are aggressive solid tumours and a paradigm of how basic and clinical research have significantly improved patient prognosis. Nevertheless, despite great success with targeted and immune-based therapies, sustained clinical responses are still limited. Moreover, the field lacks molecular markers of diagnosis, and the knowledge about how melanomas progress is largely incomplete. One of the main objectives of our Group is to define modulators of this aggressive behaviour. In particular, we are interested in identifying mechanisms that drive (pre) metastatic niche formation *in vivo*, specifically those acting in a systemic manner already from early stages of melanoma development, creating “permissive” microenvironment(s) for tumour progression.

Our Group’s main aims are to:

- define when and how melanomas act “at a distance” (on stromal and immune compartments) before tumour cell dissemination.
- determine how melanoma cells evade the immune system, and whether distinct mechanisms may be activated at different anatomical sites.
- develop anticancer agents to prevent and eliminate metastatic sites.

New immune suppressors that favour melanoma progression

One of the long-term objectives of the Melanoma Group is to discover **new melanoma drivers**. We previously identified a cluster of endolysosomal-associated genes that distinguish melanoma from over 35 additional malignancies (Alonso-Curbelo *et al.*, *Cancer Cell* 2014). Further analyses of lysosomal-dependent pathways also revealed distinctive features of autophagy genes (ATG5) and RNA binding proteins (CPEB4, CELF1 and IGF2BP1) with selective roles in melanoma (García-Fernández *et al.*, *Autophagy* 2016; Perez-Guijarro *et al.*, *Nat Commun* 2016; Cifdaloz *et al.*, *Nat Commun* 2017; Karras *et al.*, *Cancer Cell*, 2019). All these proteins had potent autocrine effects on the tumour cells where they were expressed. However, we were also interested in melanoma-secreted factors that could exert long-range activities at visceral organs, particularly in the generation of premetastatic niches.

Our Group pioneered the analysis of such systemic effects *in vivo* by exploiting melanoma “MetAlert” mice, which have the unique feature of visualising tumour-activated lymphatic vasculature (Olmeda *et al.*, *Nature* 2017). “MetAlert” animals, in combination with human tissue specimens, revealed the

growth factor MIDKINE (MDK) as a new driver of melanoma metastasis. We have now performed loss- and gain-of-function studies of downstream effectors of MDK *in vitro* and *in vivo* (mouse xenograft models), combined with expression studies in large patient cohorts. These studies have revealed yet a new function of MDK in immune suppression. Specifically, we identified a MDK-associated gene set that was able to separate melanoma patients with a differing transcriptomic profile, involving in particular a variety of immunomodulators (Cerezo-Wallis *et al.*, *Nature Medicine* 2020). Curiously, although MDK promoted an inflammatory secretome (driven in part by NF- κ B), the ultimate outcome was an immunotolerant microenvironment whereby macrophages are recruited to tumours, but instead of attacking the cancer cells, promote a dysfunctional state in CD8+ T cells, ultimately favouring immune escape (FIGURE 1, left part). This “Jekyll and Hyde syndrome” described above, whereby the immune system can shift from an anti-tumoural to a pro-tumoural phenotype depending on MDK expression, was recently published in *Nature Medicine* (Cerezo-Wallis *et al.*, 2020) and featured on the cover of the journal.

Gene signatures that define response to immune checkpoint blockade in melanoma patients

Having found that MDK promoted immune suppression, our next approach was to block its function genetically or pharmacologically. Using various murine systems, we found that MDK inhibition favoured the response to vaccination treatments, and importantly, promoted an interferon (IFN)-driven secretome that enhanced the effect of immune checkpoint blockers (ICB) (summarised in FIGURE 1, right part). This IFN-signalling resulting from MDK blockade was enriched in 6 independent clinical cohorts of melanoma patients treated with ICB (see examples in FIGURE 2). Therefore, these results provided proof of principle for MDK inhibition as a strategy to prime immunologically unresponsive tumours into “hot” lesions with an improved response to ICB. The novelty and physiological relevance of these data received considerable attention in the media (TV, press, radio) and were echoed in independent News & Views in *Nature Reviews Cancer*, *Cancer Discovery* and in *Pigment Cell and Melanoma Research*. ■

FIGURE 1 Immune suppressive roles of Midkine in melanoma progression and metastasis. Summary of MDK-dependent polarisation of macrophages towards tolerogenic phenotypes that ultimately lead to T cell dysfunction (from Cerezo-Wallis *et al.*, *Nature Medicine* 2020).

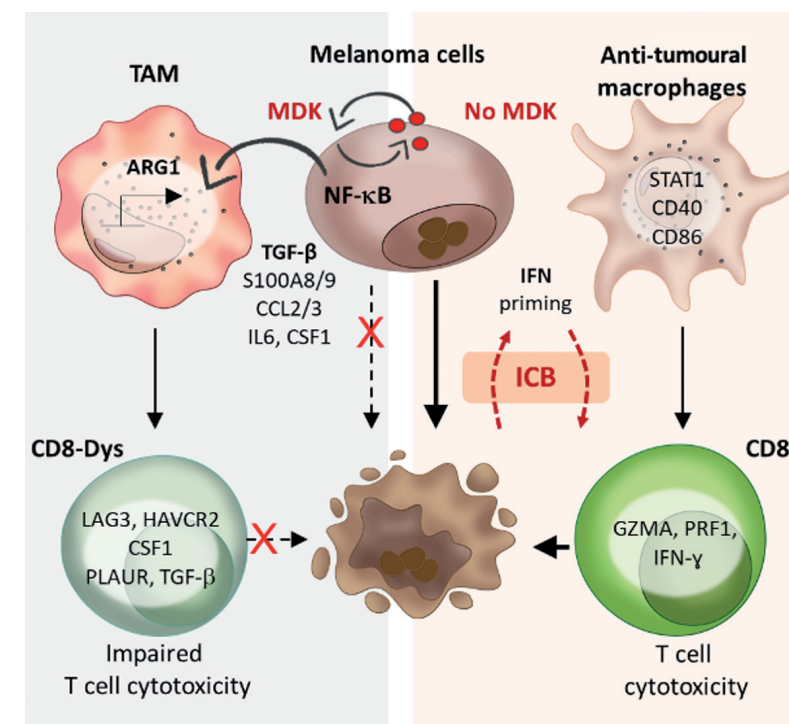
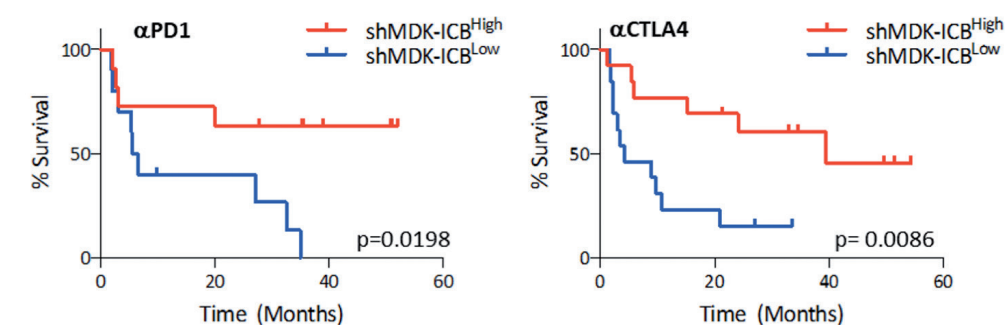


FIGURE 2 Therapeutic implications of dual inhibition of MDK and immune checkpoint blockade. Differential survival of melanoma patients treated with anti-PD1 or anti-CTLA4 antibody depending on whether or not their expression profile is enriched in a gene signature identified by dual blockade of MDK and ICB in mouse xenograft models (here labelled as shMDK-ICB^{High} and ^{Low}, respectively).



PUBLICATIONS

- Cerezo-Wallis D, Contreras-Alcalde M, Troulé K, Catena X, Mucientes C, Calvo TG, Cañón E, Tejedro C, Pennacchi PC, Hogan S, Kölblinger P, Tejero H, Chen AX, Ibarz N, Graña-Castro O, Martínez L, Muñoz J, Ortiz-Romero P, Rodríguez-Peralto JL, Gómez-López G,

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• Lund AW, Soengas MS (2020). Lymph: (Fe)rryng melanoma to safety. *Cancer Cell* 38, 446-448.

- Patton E *et al.* (incl. Soengas MS) (2020). Melanoma models for the next generation of therapies. *Cancer Cell*. PMID: 33545064.
- Atkins M *et al.* (incl. Soengas MS) (2020). The state of melanoma: emergent challenges and opportunities. *Clin Cancer Res*. PMID: 33414132.

AWARDS AND RECOGNITION

- Vicepresident/President Elect of ASEI-CA, the Spanish Association for Cancer Research.
- ERC-Advanced Grant METALERT-STOP, European Research Council.