Local Inhibition of MEK/Akt Prevents Cellular Growth in Human Congenital Melanocytic Nevi

Thomas Rouillé[1], Selim Aractingi[1,2], Natacha Kadlub[3], Sylvie Fraitag[4], Alexandre How-Kit[5], Philippe Moguelet[6], Arnaud Picard[3], Romain H. Fontaine[1], Sarah Guégan[1,2]

[1]INSERM UMRS 938, Sorbonne Université, Paris; [2]Dermatology, Hôpital Cochin, AP-HP, Paris; [3]Plastic Surgery and [4]Pathology, Hôpital Necker-Enfants-Malades, AP-HP, Paris; [5]Laboratory for Functional Genomics, Fondation Jean Dausset – CEPH, Paris; [6]Pathology, Hôpital Tenon, AP-HP, Paris, France.

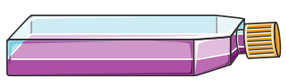
INTRODUCTION

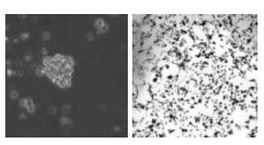
Large congenital melanocytic nevi (*l*CMN) are benign melanocytic tumors associated with an increased risk of melanoma transformation. They harbour predominantly a post-zygotic somatic *NRAS* mutation [1,2]. Management is based exclusively on iterative surgical procedures in the absence of validated medical therapy. The aim of our study was to analyze in preclinical models of lCMN the impact of an intra-lesional medical treatment targeting signaling pathways downstream of NRAS.

METHODS

Seventeen patients harboring *NRAS*-mutated lCMN were included. MEK and AKT inhibitors were tested alone or in combination in different *in vitro, ex vivo* and *in vivo* assays using patient-derived nevocytes or CMN tissue.

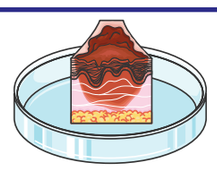
In vitro
Patient-derived lCMN nevocytes

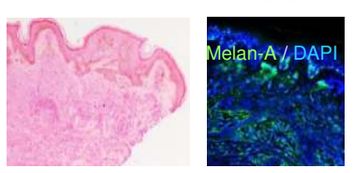




Proliferation, cytotoxicity, Western Blot

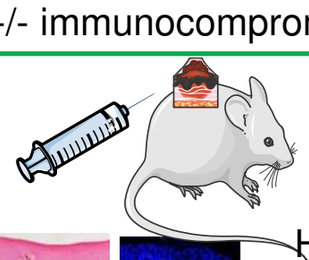
Ex vivo
lCMN tissue explants cultured for 5 days

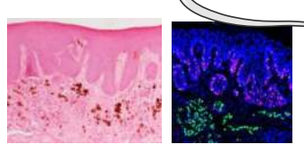




Histology, immunohisto-chemistry

In vivo
Full thickness lCMN xenografts in Rag2-/- immunocompromised mice





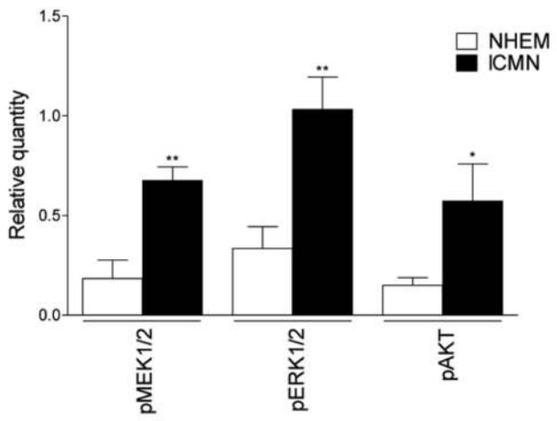
Histology, immunohisto-chemistry

RESULTS

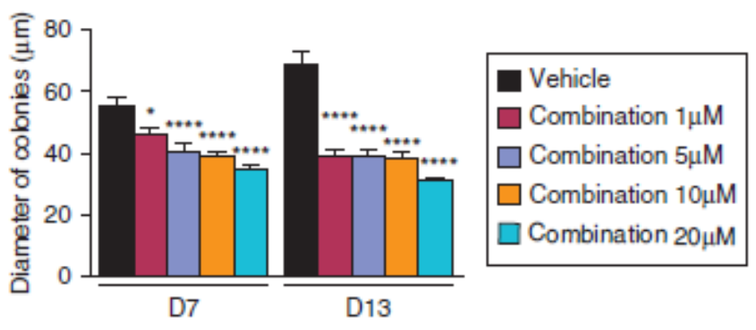
Nevocytes obtained from lCMN displayed an overactivation of MAPK and AKT pathways when compared to primary melanocytes NHEM. MEK (Binimetinib) and AKT (GSK690693) inhibitors reduced nevosphere diameter in sphere-forming assays, as well as nevocyte cell viability and proliferation in *in vitro* assays (A). Standardized lCMN explants were then cultured *ex vivo* with the same inhibitors which induced a decrease in MelanA+ and Sox10+ cells in both epidermis and dermis (B). A similar reduction in melanocyte cell numbers was not observed in control normal skin explants. Finally, intradermal injections of these inhibitors were performed within standardized lCMN xenografts in Rag2$^{-/-}$ mice. They induced a dramatic decrease in nevocytes in treated xenografts which persisted 30 days after the end of treatment (C). Melanocytes in control normal skin xenografts were not affected by a similar treatment.

A

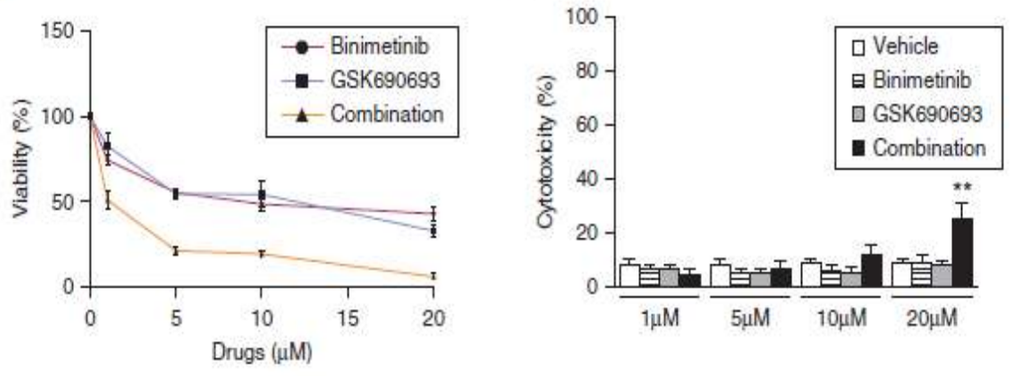
Western blot analysis



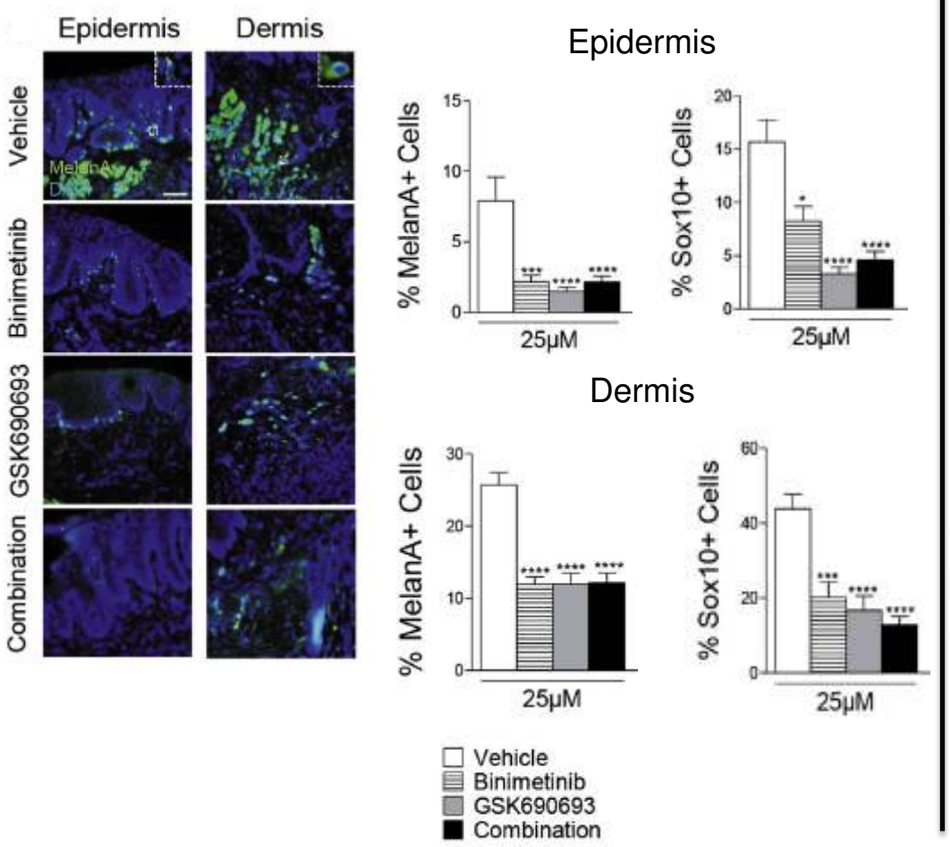
Sphere forming assay



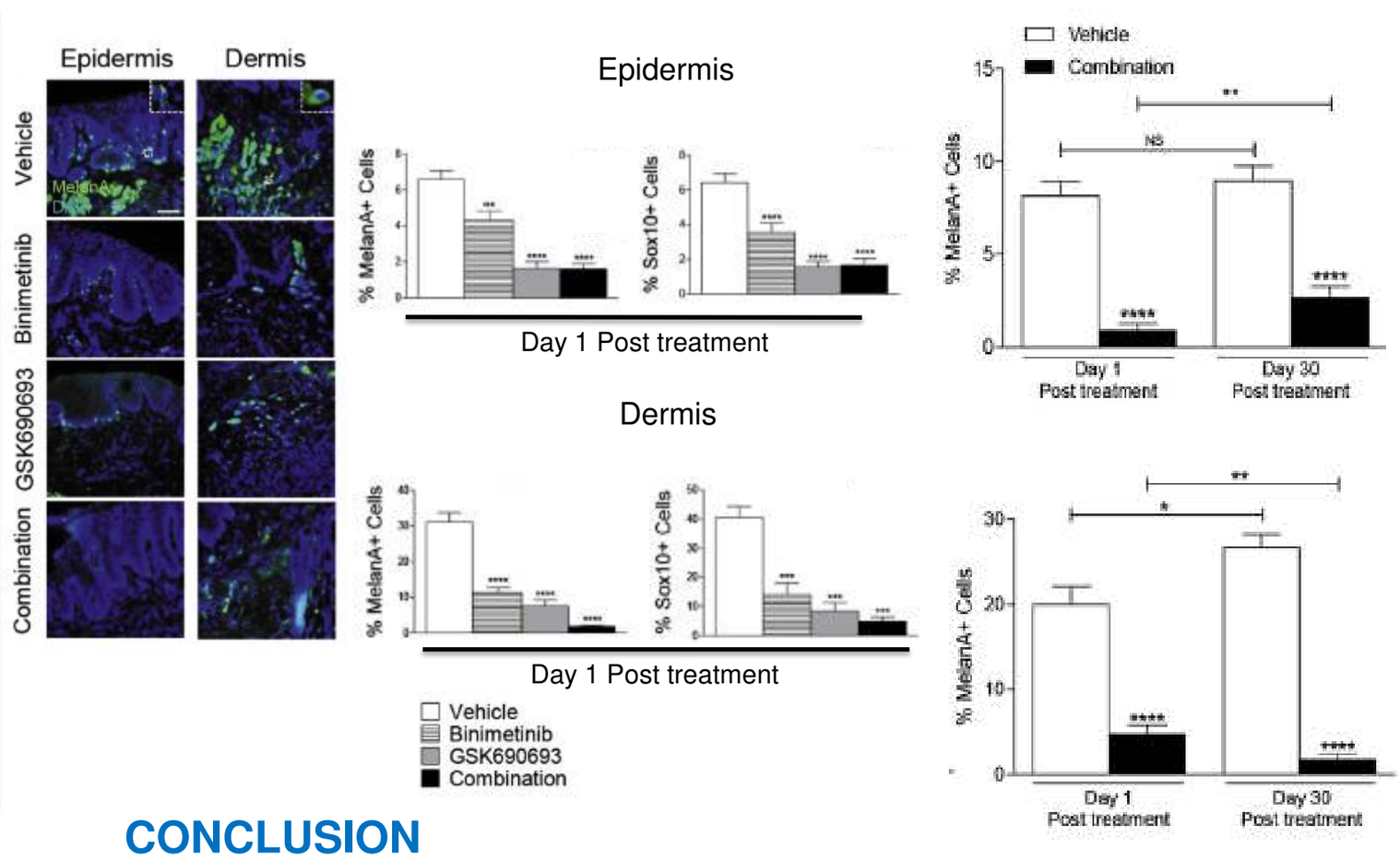
Proliferation and cytotoxicity assays



B Decrease in nevocyte numbers in lCMN explants following MEK/Akt inhibition



C Decrease in nevocyte numbers in lCMN xenografts following MEK/Akt inhibition



CONCLUSION

Using nevus explant and xenograft preclinical models, we demonstrated that intradermal MEK/AKT inhibition might serve as neo-adjuvant therapy for the treatment of NRAS-mutated CMN to avoid iterative surgeries. Additional preclinical and clinical studies are of course needed.

1 Kinsler et al, J Invest Dermatol 2013 ; 2 Charbel et al, J Invest Dermatol 2014