DEAR EDITOR, Dystrophic epidermolysis bullosa (DEB) is a skin- blistering disease caused by mutations in the COL7A1 gene encoding the anchoring fibril-constituent collagen VII.1 Secondary to skin fragility, DEB manifests as chronic wounds and progressive soft tissue fibrosis. As a consequence of a chronically injured and stiffened dermal microenvironment people with severe DEB are prone to developing early-onset metastatic cutaneous squamous cell carcinomas (cSCCs).1,2 Dermal fibrosis in DEB is paradigmatic of injury- and inflammation-driven activation of fibrogenic processes (Nyström and Bruckner-Tuderman, and references therein).2 Transforming growth factor (TGF)-β and interleukin (IL)-6 have been suggested to mediate fibrosis in DEB.1,3 The Janus kinase (JAK)1/2–signal transducers and activators of transcription 3 (STAT3) signalling pathway is a prominent downstream conductor of...(continued)

Fig 1. STAT3 targeting in DEB. (a) Keratin 14 (green) staining of sections of three-dimensional organotypic cocultures composed of DEB cSCC keratinocytes and DEB fibroblasts treated with control (DMSO) or RXL (1 μmol L⁻¹) for 3 weeks. (b) Left: quantification of the macroscopic wound healing in DEB mice with or without RXL treatment. Middle: quantification of the epithelial tongues (six wounds per group) in DEB mice with or without RXL treatment. Right: staining of α-SMA+ (red) fibroblasts (myofibroblasts) in the dermis 9 days after wounding. Myofibroblasts were lost from control wounds but remained abundant in RXL-treated wounds, suggesting delayed wound contraction. (c) Outcome of RXL treatment of eraser-induced blisters and dermal fibrosis in DEB mice. Left: PRS visualized under polarizing light of sections from repeatedly blistered back skin treated with RXL or DMSO for 30 days. Turquoise line shows the outer surface of the skin. Right: quantification of the stained area (n = 3 per group). (d) Outcome of RXL treatment on naturally progressing forepaw fibrosis in DEB mice. Left: RXL- and DMSO-treated forepaws at the start and after treatment for 80 consecutive days. Middle: PRS of forepaws after treatment with RXL or DMSO for 80 days. Right: quantification of the stained area (n = 3 per group). The mouse studies were approved by the regional ethics review board (Regierungspräsidium Freiburg) approval numbers: G14/90, G14/93 and G16/17. cSCC, cutaneous squamous cell carcinoma; DAPI, 4',6-diamidino-2-phenylindole; DEB, dystrophic epidermolysis bullosa; DMSO, dimethyl sulfoxide; PRS, picrosirius red staining; RXL, ruxolitinib; SMA, smooth muscle actin; STAT3, signal transducers and activators of transcription 3; blue, DMSO treatment, red, RXL treatment. * p < 0.05; ** p < 0.001, mean ± SEM.
IL-6 signalling and its activity contextually intersects with TGF-β signalling. The anti-inflammatory activities of JAK1/2–STAT3 targeting in skin are clinically well established. However, paradoxically, selective STAT3 activation in macrophages may protect against fibrosis. Thus, the pro- and antifibrotic effects of STAT3 targeting seem strongly context-dependent and cannot be generalized for all conditions and settings.

Staining of human DEB skin revealed enhanced phospho-Tyr 705 STAT3 (pSTAT3) in both the epidermis and the dermis (not shown). Dermal STAT3 activity posited it to be involved in profibrotic processes. Consequently, we targeted JAK1/2–STAT3 in dermal fibroblasts with the clinically approved JAK1/2 inhibitor ruxolitinib (RXL, Jakavi). In DEB fibroblasts (from four donors with molecularly confirmed complete collagen VII deficiency) RXL potently and dose-dependently reduced pSTAT3 and contraction of fibroblast-populated free-floating collagen lattices (not shown).

In immortalized DEB keratinocytes – representing early events of oncogenesis – 0.5 μmol L⁻¹ RXL suppressed expression of multiple genes linked to oncogenesis and immune-evasion including programmed death ligand 1/CD274 (not shown). Because fibrosis-associated stromal remodelling promotes DEB cSCC progression we evaluated the ability of RXL to counteract invasion in organotypic cocultures of DEB cSCC keratinocytes and DEB fibroblasts. In this system, RXL significantly reduced cSCC keratinocyte invasion (Fig. 1a). Collectively, our in vitro data suggested that targeting the JAK1/2–STAT3 axis could have synergistic benefits on fibrosis and cSCC progression in DEB.

The collagen VII hypomorphic mouse (the DEB mouse) faithfully recapitulates most signs of severe human DEB, including progressive fusion of the digits. Histological and biochemical analyses confirmed increased pSTAT3 in DEB mouse skin (not shown). Thus, this mouse represents an adequate model to assess STAT3-targeting therapy for DEB in vivo. A potential DEB therapy should not obstruct wound healing. For RXL, this was a particular concern as epidermal JAK1/2–STAT3 activity is essential for physiological re-epithelialization, and downstream epidermal growth factor receptor targeting delays healing of DEB wounds. To study the effect on wound healing, excision wounds in DEB mice were topically treated with RXL 15 mg mL⁻¹ daily. RXL alleviated inflammation (not shown) but delayed macroscopic wound healing; this appeared to be a combined outcome of delayed re-epithelialization and myofibroblast formation (Fig. 1b).

To address the fibrosis-inhibiting potential of RXL, we then tested its effect on injury-induced dermal remodelling in a controlled setting. To evoke fibrosis, we applied standardized frictional challenges by rubbing shaved back skin with an eraser until blisters appeared. After blister induction, 15 mg mL⁻¹ RXL was applied daily at the blister site. Three days later the same site was rechallenged: blisters reappeared but the challenge did not induce wounds; the cycle was repeated nine times. After 30 days of treatment, the scars of RXL-treated healed blisters were less prominent, and histological analyses confirmed efficient pSTAT3 reduction (not shown). Picrosirius red stained skin sections supported the macroscopic observation (Fig. 1c). Thus, in the setting of induced and expedited fibrosis, a relatively short treatment with RXL protected against dermal fibrotic remodelling.

Lastly, to evaluate the benefit of RXL on naturally progressing DEB fibrosis we followed formation of forepaw deformities. RXL 25 μL (15 mg mL⁻¹) was applied daily on one forepaw for 80 days; the other forepaw was treated with dimethyl sulfoxide only. The mice did not tolerate this treatment well; their general health deteriorated and five of six mice had to be killed prematurely. Despite efficient pSTAT3 reduction, RXL did not prevent fusion of digits (Fig. 1d).

In conclusion, targeting the JAK1/2–STAT3 axis by RXL did not result in unequivocal changes in DEB. In vitro fibroblast activation and tumour progression were reduced, but in vivo RXL had a limited effect on progressive DEB-associated fibrosis and, importantly, it delayed wound healing. This emphasizes the need for relevant models and careful preclinical assessment of potential symptom-relief drugs.

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Clinical significance of BRAF/NRAS concurrent mutations in a clinic-based metastatic melanoma cohort

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Dear Editor, Innovative therapeutic strategies in metastatic melanoma depend on molecular features and the combination of BRAF and MEK inhibitors (BRAFi+MEKi), which has recently shown an improved clinical efficacy in patients with BRAFV600MUT metastatic melanoma, is now the standard of care. BRAF and NRAS activating mutations lead to mitogen-activated protein kinase (MAPK) pathway deregulation and may coexist despite being described previously as being mutually exclusive. This event is probably related to the existence of several mutually exclusive subclones corresponding to a mixture of singly mutated melanoma cells or may have arisen as shown by in vitro treatment. A study of 3399 melanomas highlighted the co-occurrence of BRAFV600E/RAS mutations in 1-5% of primary tumours and 5-6% of metastatic lesions. Moreover, clonal evolution, described in acquired resistance to BRAFi, involved NRAS upregulation in MAPK pathway reactivation. Hence, considering tumour heterogeneity is essential for therapeutic and clinical management. From 2009 to 2017, 651 patients with metastatic melanoma, genotyped as part of their routine care at the Oncodermatology Centre of Saint-Louis Hospital (Paris, France), harboured a BRAFV600 mutation. Among them, 21 BRAFi±MEKi-treated patients had at least one sample harbouring BRAF/NRAS concurrent mutations (n = 2 primary tumours and n = 19 metastases) and were included in this retrospective study after signed informed consent. Clinical response was evaluated using RECIST V1.1. All patients harboured a BRAFV600 mutation before targeted therapy initiation and showed disease progression. NRAS genotyping in available tumour samples at baseline or during follow-up revealed a mutation on codons 61 and 12/13 in 18 (86%) and three (14%) patients, respectively. Fifteen patients had multiple samples (Fig. 1a). Among the 21 patients, 18 had an available baseline genotype: six NRASWT at baseline harbouring an NRASMUT after targeted therapy initiation, and 12 NRASMUT at baseline that at follow-up genotyping remained NRASMUT for two patients and became NRASWT for three (seven patients had no available follow-up sample). To provide arguments regarding the potential clinical impact of co-occurrence of BRAF/NRAS mutations, patients with NRASWT (n = 6) and NRASMUT (n = 12) baseline tumours were compared. Similar baseline clinical characteristics were retrieved (superficial spreading melanoma subtype, P = 0.64; mean age at therapy initiation, P = 0.50; American Joint Committee on Cancer (AJCC) stage IV1Mc, P = 0.27; brain metastasis, P = 1.00; elevated lactate dehydrogenase level, P = 1.00; BRAFi±MEKi as first-line therapy, P = 0.63). Median progression-free survival (PFS), defined as the time from therapy initiation to disease progression or death whichever occurred first, was 7.4 [95% confidence interval (CI) 4.0 to not available (NA)], and 2.8 (95% CI 2.0 to NA) months for baseline NRASWT and NRASMUT group, respectively. Although not statistically significant (P = 0.32, log-rank test), this trend highlighted a different clinical course according to NRAS baseline status. To ensure relevance of our findings, the same analysis was conducted on patients treated with BRAFi only. The observed trend persisted and a better PFS was retrieved in the baseline NRASWT group vs. the NRASMUT group.

Two patients had blood samples at baseline and during follow-up allowing the detection of BRAF and NRAS mutations in circulating tumour DNA (ctDNA) using E-ice-COLD-PCR (0.1% sensitivity threshold). In patient 5, BRAFV600E ctDNA remained low after dabrafenib initiation and increased after disease progression suggesting a resistance mechanism independent from NRAS mutations. In patient 10, NRASQ61K ctDNA variation mirrored tumour response, and the increase in allelic frequency during follow-up coincided with tumour progression (Fig. 1b).

This retrospective study presents the clinical course in a real-life setting of concurrent BRAF/NRAS mutations in a small cohort of patients with melanoma and highlights two profiles. At baseline, patients are either NRASMUT or NRASWT with a NRAS mutation detected under targeted therapy. These results depict the tumour heterogeneity with different mutational status depending on tumour sites and the capacity of evolution of the mutational profile. Regarding the six patients with NRAS mutation occurring after therapy initiation, we can assume that a resistance mechanism could be introduced by NRAS-dependent MAPK pathway reactivation. These six patients present a PFS comparable to that observed in a retrospective study of about 60 patients with metastatic melanoma BRAFMUT under BRAFi±MEKi. Despite the lack of significance and the small size of our cohort requiring further analyses, patients with a NRASMUT baseline tumour sample seem to have a worse PFS, suggesting a role of NRAS mutations as a primary mechanism of resistance. Larkin et al. previously analysed several baseline concurrent mutations with BRAFV600MUT and did not retrieve any association with PFS. This can be explained by the fact that NRAS mutations were not considered separately from other RAS/RAF/RTK mutations. In this context, identifying patients with metastatic melanoma with BRAF/NRAS concurrent mutations appears to be a crucial point prompting the implementation of a particular