

Emerging Therapies for Congenital Melanocytic Nevus

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Abstract

Congenital melanocytic nevi (CMN) are benign melanocytic neoplasms that vary in genetic drivers leading to the formation of pigmented lesions. Activation of Mitogen-Activated Protein Kinase (MAPK) pathway members through oncogenic mutations of *NRAS* or *BRAF* and more rarely by gene fusions allow expansion of nevomelanocytes to form CMN. Pre-clinical approaches to CMN therapy are expanding with the use of small-molecular inhibitors to mitogen-activated protein kinase kinase (MEK), RNA-based therapeutic approaches, and leveraging topical immunotherapy to regress nevi using *in vitro* and *in vivo* models and select case reports. Continued identification of CMN drivers combined with emerging therapeutic strategies will help improve CMN patient response and outcomes in the future.

Keywords: Congenital Melanocytic Nevus (CMN), *NRAS*, *BRAF*, Mitogen-activated Protein Kinase (MAPK) pathway, MAPK Targeted Therapy, RNA-based Therapy, Topical immunotherapy, Trametinib, Binimetinib, SADBE

Introduction

Congenital melanocytic nevi (CMN) are benign proliferations of melanocytes that form pigmented lesions on the skin *in utero*. The nevomelanocytes that make up a nevus lack dendritic processes, differ in skin location, and present as nests of cells rather than individual melanocytes. CMN arise during embryonic development and are driven by activating mutations of *NRAS*, 61-95% of cases across multiple studies [1-3], activating *BRAF* mutations, 7-15% of cases, and more rarely *BRAF* fusions [1-4]. Current therapies used to treat CMN include surgical excision, pigment-specific/ablative laser therapy, and cryotherapy as shown in **Table 1** [5-7]. These standard of care treatments for CMN often result in incomplete removal, dyspigmentation, repigmentation, and scarring [5-7], which can additionally complicate accurate clinical monitoring for the risk of malignant transformation. Studies show that CMN patients struggle with the impact of nevi on their quality of life, which may deteriorate undergoing procedures like tissue expansion or if results are incomplete or temporary [8]. Assessment of quality of life before and after surgical resection of CMN is an ongoing concern and the subject of an open clinical trial (NCT02280889). Recent studies have developed

new systems using CMN patient-derived cell lines, human xenograft models, and genetically engineered murine models to characterize causative drivers and signaling pathways while providing tools to test therapeutic strategies that regress nevi as seen in **Figure 1** [4,9,10]. New therapies for CMN including small molecular inhibitors, RNA-based therapy, and topical immunotherapy show promise in regressing nevi in preclinical models and may improve the quality of life for CMN patients.

Driver mutations in MAPK-Driven Congenital Melanocytic Nevus

Many driver mutations in CMN lead to activation of the mitogen-activated protein kinase signaling pathway (MAPK) [4,9,10]. This pathway responds to growth signals through the activation of *NRAS*, starting a kinase cascade that activates *BRAF* to phosphorylate mitogen-activated protein kinase kinase (MEK) which in turn phosphorylates extracellular signal-regulated kinase (ERK) to regulate transcription factors that promote cell growth and proliferation [11]. Constitutive signaling through the MAPK pathway caused by oncogenic mutations of *NRAS* or *BRAF* drive CMN promoting cell cycle progression, differentiation, and proliferation giving rise to

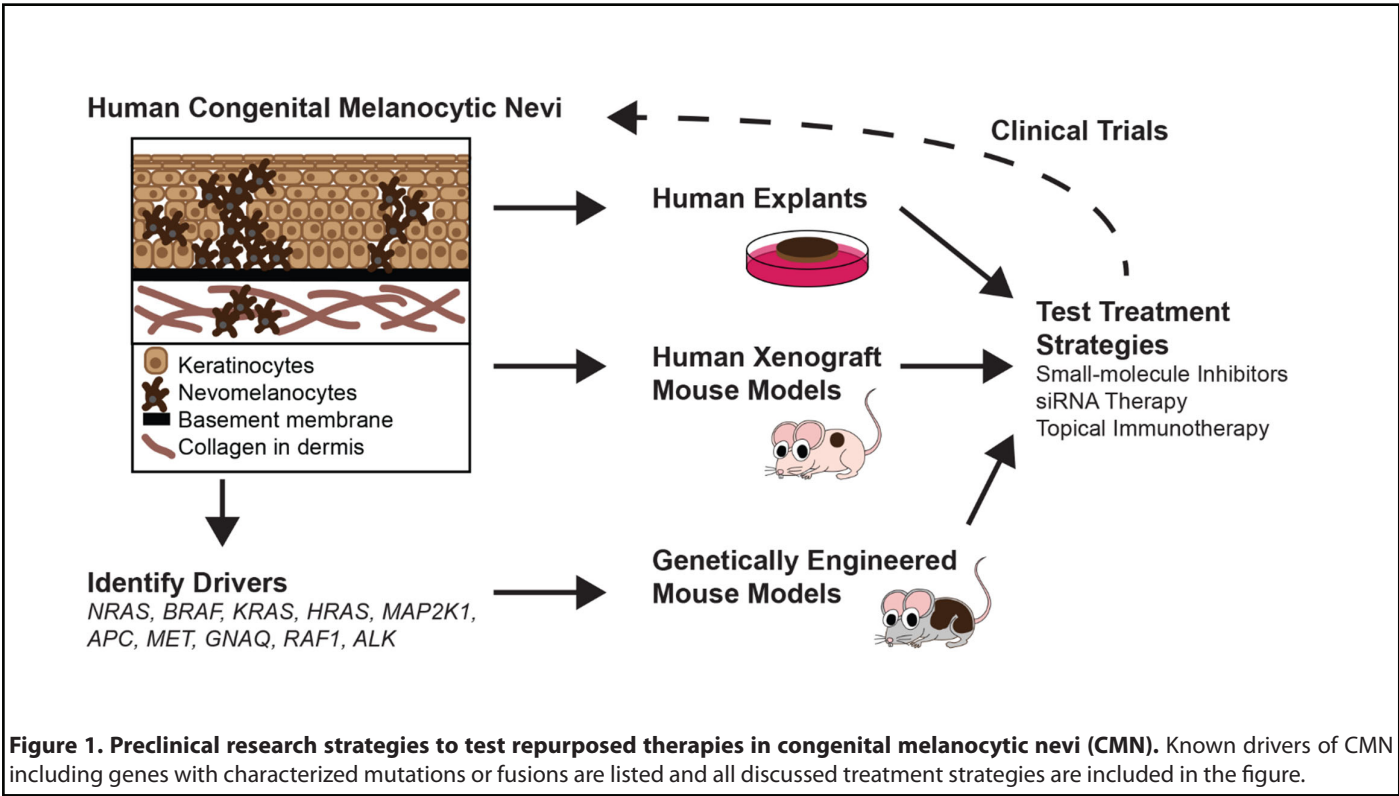


Figure 1. Preclinical research strategies to test repurposed therapies in congenital melanocytic nevi (CMN). Known drivers of CMN including genes with characterized mutations or fusions are listed and all discussed treatment strategies are included in the figure.

hyperpigmented lesions on the skin [12]. The proliferation phase of nevi is blocked by oncogene-induced senescence that prevents progression to melanoma as proliferative nevus cells lose their ability to expand [12,13]. Even though CMN transformation to melanoma is infrequent (1-5% of cases) [8,14], there is a long-established link between CMN patients and childhood melanoma with the highest risk associated with the largest lesions [15,16].

Mutations in *NRAS* are most common in CMN patients. Across multiple studies of CMN with *NRAS* mutations, 70-74% were driven by Q61K mutations, another 21-27% by Q61R, and additional mutations occurring in 1-2% of cases [2,3,17]. Both *NRAS*^{Q61K} and *NRAS*^{Q61R} mutations lead to elevated levels of MAPK signaling in human nevi and mouse models [9,10,18]. The frequency of *NRAS* driven CMN is enriched in nevi with a projected adult size over 60 cm [17].

The second most mutated gene in CMN is *BRAF* with *BRAF*^{V600E} found in 92-100% of *BRAF*-mutant lesions [2,3,17]. With few other pathological differences, *BRAF* mutant CMN are commonly associated with a distinctive nodular phenotype [17]. *BRAF*-driven CMN also occurs following chromosomal rearrangements when loss of the *BRAF* autoinhibitory domain results in the tyrosine kinase domain regulated by a fusion gene partner [3,4]. These fusion-driven nevi have shown levels of MAPK signaling that are similar compared with other CMN oncogenic drivers [4]. Some *BRAF* fusion driven CMN patients do have chronic intractable pruritus [4], which highlights differences in MAPK activation can alter severity of CMN.

Additional driver mutations and fusion proteins have been identified in smaller proportions of CMN patients including multiple mutations within the *MAP2K1* gene, *KRAS* alterations, *HRAS* mutations, *MET* alterations, *APC* mutations, *GNAQ* alterations, *ALK* fusions and oncogenic *RAF1* fusions [2,19-21] indicating that aberrant MAPK signaling is a key step in congenital nevi formation. Various studies have also identified that 4-24% of CMN lesions have unknown drivers [2,4,19] which will hopefully lead to the continued identification of additional novel CMN drivers. While there is a growing library of identified CMN driver mutations, patients without an identified driver mutation need to be considered in developing new screening methods and therapeutic strategies for CMN.

Developments in Precision Therapy

New therapeutic strategies for CMN have taken multiple approaches from small molecule inhibitors to RNA-based therapies and immunotherapy summarized in Table 1. The rise of small molecule inhibitors in cancer biology has led researchers to investigate the effectiveness of targeted therapies in benign lesions. Due to the activation of the MAPK pathway, initial efforts in targeted therapy have focused on MEK inhibitors (Binimetinib and Trametinib) clinically approved to treat patients with *BRAF* mutant melanoma [22], with manageable cutaneous side effects including pruritus and maculopapular rash [23]. In murine models, Trametinib regressed *NRAS*^{Q61R}-driven CMN with reduction of MelanA-positive melanocytes and melanin by Fontana-Masson staining in treated skin as a single-agent and in combination with the

Table 1. Clinical and pre-clinical therapies for congenital melanocytic nevi (CMN). Pre-clinical uses and desired outcomes in the context of CMN treatment. N/A indicates current clinical therapies that are not in pre-clinical stages for CMN. Abbreviations: BCC: Basal Cell Carcinoma; SCC: Squamous Cell Carcinoma; MAPK: Mitogen-Activated Protein Kinase; SADBE: Squaric Acid Dibutylester.

Therapy	CMN Drivers	Clinical Use	Pre-Clinical Use (CMN)	Desired Outcomes (CMN)	Side Effects	Citations
Surgery	CMN in operable locations	CMN CMN with melanoma progression	N/A	Removal of nevus through excision	Cosmetic dissatisfaction, Hypertrophic scarring, Restrictions in joint mobility, Impartial Removal	[7]
Laser Therapy (Pigment specific and Ablative)	CMN in operable locations	CMN, Freckles, Sunspots, Tattoo	N/A	Clearance of hyperpigmentation	Scarring, Textual changes, Dyspigmentation, Repigmentation, Wound infection, Short-term clearance	[6]
Cryosurgery	All CMN	CMN, Lentigines, Seborrheic keratosis, Actinic keratosis, Skin Tags, Warts, BCC, SCC	N/A	Necrotize nevus tissue, Reduce CMN bulk	Edema, Blistering, Bleeding, Paresthesia, Tendon rupture, Alopecia, Scarring, Hypopigmentation, Atrophy, Pseudomelanoma	[5]
MAPK-Targeted Therapy	Activating mutations of NRAS and BRAF, BRAF fusions	CMN, Melanoma	Murine models, Human CMN tissue	Depigmentation, reduce growth and proliferation of nevus cells	Maculopapular rash, pruritus	[4,10,23-26]
RNA-based Therapy	NRAS driven CMN	Hereditary transthyretin amyloidosis	Human CMN tissue	Depigmentation, ablation of pigment producing cells	Off-target gene silencing, erythema, infusion reactions	[9,29-31]
SADBE	NRAS driven CMN	Alopecia areata, Warts	Mouse models, Human xenografts	Depigmentation, Loss of melanocytes, recruitment of macrophages	Erythema, Inflammation, Skin irritation, Pruritus	[10,32-34]

PI3K inhibitor Omipalisib [10]. Isolated cultures from large and giant CMNs driven by multiple *NRAS* mutations are sensitive to both Binimetinib and AKT inhibition, with the combination significantly improving cytotoxicity of melanocytes in cultured explants where the percent of MelanA-positive melanocytes within the explant was reduced from 26% in vehicle-treated controls to 12% when treated with the Binimetinib and AKT inhibitors [24]. Individual reports of patients with severe complications related to *NRAS*^{Q61K} and *BRAF* fusion-driven CMN, including neurocutaneous melanosis, melanoma, or behavior-altering pruritus, have demonstrated reduction of symptoms following Trametinib treatments [4,25,26]. Due to short treatment windows, additional clinical data are needed to measure the long-term response to MEK inhibitors and other small-molecule inhibitors in CMN patients to assess nevi

regression and melanocyte removal. Melanoma studies have found that combination treatments with *BRAF*^{V600}-mutant inhibitors (vemurafenib, dabrafenib, or encorafenib) increase the sensitivity of *NRAS*-mutant cell lines to MEK inhibition (trametinib, binimetinib, or cobimetinib) [27], expanding the use of *BRAF* inhibitors to contexts without mutant *BRAF*. Active research may also include the development of pan-RAF inhibitors. In addition, more MAPK inhibitors are being developed including Ulixertinib targeting ERK where partial tumor responses were observed in *NRAS* and *BRAF* mutant melanoma patients, including some that had previously been treated with *BRAF*^{V600E} and MEK inhibitors [28]. Taken together these findings highlight the potential of small-molecule inhibitors and of combination therapy to treat CMN patients.

RNA therapy is another developing therapeutic strategy for CMN. Silencing RNA therapies utilize small interfering RNA (siRNA) oligonucleotides to target specific messenger RNA transcripts and degrade the target protein. Clinically, siRNA therapies against transthyretin are approved to treat hereditary transthyretin amyloidosis for patients presenting with neuropathy symptoms [29]. Clinical trials found that the siRNA therapy Patisiran reduced transthyretin levels in the blood and improved life expectancy of patients with side effects including erythema and infusion reactions [30,31]. Utilizing siRNA targeting *NRAS*^{Q61K}, nevus cells exhibited approximately 40% reduced MAPK signaling while undergoing apoptosis in both patient derived CMN cell lines and a *NRAS*^{Q61K} mouse model [9]. Combination *siNRAS*^{Q61K} with Trametinib improved inhibition of proliferation from 50% to 95% of cells failing to incorporate 5-ethynyl-2'-deoxyuridine during replication and Endoplasmic Reticulum-stress induced apoptosis initiation via significant increases in caspase 3/7 activity [9]. The combination of a clinically approved technique that can be repurposed for CMN with individualized siRNA targeting specific drivers would be useful for the array of known driver mutations and fusion genes and as more CMN drivers are identified. Through further studies systemic siRNA targeting of mutant *NRAS* could lead to a promising therapy for CMN patients including those individuals with systemic complications like neurocutaneous melanosis.

Another area of research for CMN therapies is topical immunotherapy treatments to regress nevi. Squaric acid dibutylester (SADBE) is a hapten molecule prescribed to treat patients with warts or alopecia who do not respond to other therapies [32-34]. SADBE can lead to erythema, blistering, and eczema [32,34] with some case reports citing hypopigmentation [35,36]. In mouse models of *NRAS*^{Q61R}-driven CMN, multiple SADBE treatments reduced gross pigmentation, loss of melanin by Fontana-Masson staining, and near complete removal of MelanA-positive melanocytes in a macrophage dependent manner [10]. This model showed an intrinsic propensity for eventual melanoma transformation—a process that was largely prevented by SADBE induced nevus regressions [10]. Similarly, human congenital nevi xenografts on immunocompromised mice (which contained macrophages) were sensitive to repeated treatments of SADBE with loss of 80% of melanocytes in treated grafts [10]. Importantly, however, since some of the nevus cells associated with CMNs are thought to reside in non-cutaneous deeper tissues (during neural crest-related migration), it may be impossible for topical treatments to achieve 100% eradication of nevus cells and melanoma risk. In contrast to targeted small-molecule inhibitors and siRNA therapy, SADBE provides a therapeutic option that may be applicable to patients without common CMN mutations in *NRAS* or *BRAF*.

Conclusions

The myriad of driver mutations and gene fusions that trigger

CMN coupled with the need for a robust therapy highlights the complexity of treating patients to regress CMN and prevent melanoma transformation. The identification of recurrent *BRAF* fusion driven CMN [4] highlights the need to expand identifiable CMN drivers including fusion products that can aberrantly activate the MAPK pathway. While the therapies discussed above have only regressed a subset of CMN drivers, the recurrent activation of MAPK signaling highlights the potential of utilizing small molecule inhibitors to regress nevi with MAPK activation [4,10,24-26]. The potential of siRNA therapy to treat CMN and achieve systemic removal of nevomelanocytes [9], will increase as additional CMN drivers are identified. SADBE has the potential to work in many CMN genetic backgrounds as a topical immunotherapy. The goal for new CMN treatment strategies is to identify single agents or combinations that lead to robust clearance of pigmented lesions and nevomelanocytes with less scarring and no repigmentation. While this is still a goal, many of the strategies discussed are already used clinically in cancer treatment (small-molecule inhibitors), hereditary transthyretin amyloidosis (siRNA therapy), and alopecia areata (topical immunotherapy) passing one hurdle in the transition from bench to clinic with dosing ranges and expected side effects established in humans. MEK inhibition and *siNRAS*^{Q61K} provide systemic therapies that may be more relevant to CMN patients with a larger nevus or systemic expansion of melanocytes, like neurocutaneous melanosis. Both siRNA therapy and SADBE may be beneficial to a wide range of patients regardless of the activating mutations, since siRNA targets can be developed towards new mutations or fusion products and SADBE triggers macrophage-mediated clearance of melanocytes. The combination of two or more of these therapies may provide a deeper armamentarium of therapeutic approaches for treatment of these patients. Research into CMN is at a crossroads with an expanding list of driver mutations and gene fusions to inform the use of model systems to facilitate the development of treatments that can improve the quality of life for CMN patients and hopefully also help to mitigate the melanoma risk.

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Author Contributions

DH: Conceptualization, Writing – Original draft preparation.
JLF: Conceptualization, Writing – Reviewing, Editing. **DEF:** Writing – Reviewing and Editing.

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