Skin Cancer: Biology & Therapy

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PATHG4500 Cellular & Molecular Biology of Cancer
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Outline

- Skin structure and types of skin cancer
- Skin cancer facts (incidence, death)
- Causes and risk factors
- Biology/Treatment (Three major types)
  - SCC
  - BCC
  - Melanoma
Skin Structure & Types of Skin Cancer

- Epidermis
- Dermis
- Hypodermis

- Hair fiber
- Pore
- Nerve ending
- Sebaceous (oil) gland
- Sensory nerve fiber
- Eccrine sweat gland
- Artery
- Vein
- Hair follicle
- Adipose tissue

- Langerhans cells
  - Keratinocytes in basal layer
  - Melanocytes

- Langerhans cell sarcoma
- Actinic Keratosis (precancerous growth)
- Squamous Cell Carcinoma (SCC)
- Basal Cell Carcinoma (BCC)
- Melanoma
- Merkel Cell Carcinoma (Rare aggressive)
> 5.4 million cases of skin cancer (NMSC) (BCC/SCC) each year in the US
- Melanoma accounts for less than 1% of skin cancer cases, but the vast majority of skin cancer deaths
- Skin cancer > the combined incidence of cancers of the breast, prostate, lung and colon
- One in five Americans will develop skin cancer in the course of a lifetime
Estimated New Cancer Cases in the US in 2017

Invasive melanoma is projected to be the 5th most common cancer for men (52,170 cases) and the 6th most common cancer for women (34,940 cases)

- The estimated 5-year survival rate for patients whose melanoma is detected early is about 98% in the US
- 62% when the disease reaches the lymph nodes
- 18% when the disease metastasizes to distant organs
Skin Cancer on the Rise

- Between 1976-1984 and 2000-2010, **BCC** incidence 145% ↑, and **SCC** 263 % ↑
- **Melanoma** rates in the US doubled from 1982 to 2011.

![Melanoma incidence graph]

- NMSC incidence rates are increasing in people younger than 40.
- Melanoma is the second most common form of cancer in females age 15-29. Its incidence is increasing faster in this age group than in males of the same age group.
Major Causes and Risk Factors
Ultraviolet (UV) Radiation: a proven human carcinogen

The vast majority of NMSCs (~90%) and melanomas (~95%) are associated with exposure to natural and artificial (tanning beds) UV light.

- The main photoproducts are formed at adjacent pyrimidines and consist of cyclobutane pyrimidine dimers (CPD), mainly thymine dimers (TTs) and pyrimidine-pyrimidone (6-4) photoproducts (6-4PPs).

- These lesions are repaired by the excision repair pathway, but when unrepaired can form the classic “UVB signature” mutations: C → T or CC → TT.

- UVA is also mutagenic, but its genetic effects mainly attribute to UVA excitation of non-DNA chromophores, resulting in reactive oxygen species-induced base oxidation to form products such as 8-oxo-7,8-dihydroguanine, as well as DNA single-strand breaks.
Excess Health Risks Associated with UV Exposure

<table>
<thead>
<tr>
<th>Exposure (No. of Studies)</th>
<th>Excess Risk (95% CI)</th>
<th>Comparison Groups</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>MELANOMA</strong></td>
<td></td>
<td></td>
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<tr>
<td>Sun exposure*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total sun exposure (N = 28)</td>
<td>34% (2, 77)</td>
<td>N/A</td>
</tr>
<tr>
<td>Intermittent sun exposure (N = 34)</td>
<td>61% (31, 99)</td>
<td>N/A</td>
</tr>
<tr>
<td>Chronic sun exposure (N = 40)</td>
<td>-5% (-13, 4)</td>
<td>N/A</td>
</tr>
<tr>
<td>Sunburn*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sunburn in childhood (N = 27)</td>
<td>91% (59, 130)</td>
<td>Ever vs never</td>
</tr>
<tr>
<td>Sunburn in adolescence (N = 13)</td>
<td>63% (42, 86)</td>
<td>Ever vs never</td>
</tr>
<tr>
<td>Sunburn in adulthood (N = 13)</td>
<td>44% (27, 63)</td>
<td>Ever vs never</td>
</tr>
<tr>
<td>Sunburn in past 5–10 years (N = 5)</td>
<td>62% (-1, 165)</td>
<td>Ever vs never</td>
</tr>
<tr>
<td>Ever sunburned in lifetime (N = 28)</td>
<td>59% (37, 83)</td>
<td>Ever vs never</td>
</tr>
<tr>
<td>Indoor tanning</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ever indoor tanned (N = 27)</td>
<td>20% (8, 34)</td>
<td>Ever vs never</td>
</tr>
<tr>
<td>Ever indoor tanned (N = 8; U.S. studies only)†</td>
<td>23% (3, 47)</td>
<td>Ever vs never</td>
</tr>
<tr>
<td>Ever indoor tanned (N = 10; studies from year 2000 onward)†</td>
<td>22% (3, 45)</td>
<td>Ever vs never</td>
</tr>
<tr>
<td>Indoor tanned before age 35 years (N = 13)†</td>
<td>59% (36, 85)</td>
<td>Ever before age 35 vs never before 35</td>
</tr>
<tr>
<td>Frequent indoor tanning (N = 15)</td>
<td>42% (15, 74)</td>
<td>Frequent vs infrequent/never</td>
</tr>
<tr>
<td>Relative risk for each indoor tanning per year (N = 4)‡</td>
<td>2% (0, 4)</td>
<td>N/A</td>
</tr>
<tr>
<td>&gt;10 lifetime tanning sessions (N = 10)‡</td>
<td>34% (5, 71)</td>
<td>&gt;10 lifetime tanning sessions vs never</td>
</tr>
<tr>
<td>Indoor tanned &gt;1 year (N = 3)‡</td>
<td>61% (-2, 167)</td>
<td>Indoor tanned &gt;1 year vs never</td>
</tr>
<tr>
<td><strong>BASAL CELL CARCINOMA</strong></td>
<td></td>
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<tr>
<td>Ever indoor tanned (N = 10)</td>
<td>29% (8, 53)</td>
<td>Ever vs never</td>
</tr>
<tr>
<td>Frequent indoor tanning (N = 4)</td>
<td>50% (-19, 177)</td>
<td>Frequent vs infrequent/never</td>
</tr>
<tr>
<td>Indoor tanned before age 25 years (N = 3)</td>
<td>40% (29, 52)</td>
<td>Ever before age 25 vs never before 25</td>
</tr>
<tr>
<td><strong>SQUAMOUS CELL CARCINOMA</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ever indoor tanned (N = 10)</td>
<td>67% (29, 117)</td>
<td>Ever vs never</td>
</tr>
<tr>
<td>Indoor tanned before age 25 years (N = 2)</td>
<td>102% (-30, 486)</td>
<td>Ever before age 25 vs never before 25</td>
</tr>
</tbody>
</table>

Increasing intermittent sun exposure in childhood and during one’s lifetime → an increased risk of all types of skin cancer.

Experiencing five or more blistering sunburns between ages 15 and 20 increases one’s melanoma risk by 80% and NMSC risk by 68%.

More than 419,000 cases of skin cancer in the U.S. each year are linked to indoor tanning, including about 245,000 BCCs, 168,000 SCCs, and 6,200 melanomas.
Pigmentary characteristics

- People with fairer skin (who often have lighter hair and eye color as well) are at increased risk

Melanoma Incidence is Highest in Countries Populated by Fair-Skinned Persons Living in High-UV Environments

South Africa has the highest incidence on the African continent, with 4.5 cases per 100,000 people per year.

United States incidence map
The highest rates of melanoma in the United States occur in the northwest and southeast states, reflecting the higher proportion of the population who are of non-Hispanic white ethnicity in those states.

Cases per 100,000 people per year
- 22.9–34.1
- 20.5–22.8
- 18.5–20.4
- 9.0–18.4
- <9.0
- No data

Europe incidence map
Switzerland has the highest incidence of melanoma in Europe, with 25.8 cases per 100,000 people per year. Southern European populations have the lowest burden of melanoma. The incidence is highest in Northern Europe, particularly in Nordic countries.

Cases per 100,000 people per year
- 16.9+
- 13.1–16.8
- 7.7–13
- 5.3–7.6
- <5.3

The highest incidence rates are found in New Zealand and Australia. The highest recorded incidence is in Queensland, Australia: 56 cases per 100,000 per year for men, and 41 cases per 100,000 per year for women.
Other Causes and Risk Factors

- **Immunosuppression**
  Among solid-organ transplant recipients (OTRs), the risk of SCC is 65 - 250X higher, and the risk of BCC is 10X higher than in the general population. Melanoma is 1.6 - 2.5x more common among OTRs.

- **Family history/Previous personal history**

- **Other environmental factors**
  Petroleum byproducts (e.g., asphalt, tar, soot, and paraffin), organophosphate compounds, and arsenic are all occupational exposures associated with cutaneous non-melanoma cancers.
  The populations in Bangladesh, Taiwan, and many other locations have high levels of skin cancer, both melanoma and non-melanoma, associated with elevated levels of arsenic in the drinking water.

- **Other radiation exposure**
  Exposure to therapeutic radiation (e.g., psoralen and UVA (PUVA) → a 3-fold to 6-fold increase in SCC).

- **Current or previous cigarette smoking**
  1.5-fold to 2-fold increase in SCC risk

- **Multiple nevi** (melanoma)

- **HPV infection**

- **Advanced Age**
Squamous Cell Carcinoma (SCC)
The development of cSCC follows the multistage model of malignant transformation.

### Initiation
- **UV/carcinogen**: irreversible genetic mutation in stem cell/progenitor cell

### Promotion
- **UV/tumor promoter**: clonal expansion of initiated cells within generalized hyperplasia
- **UV/tumor promoter**: outgrowth of pre-malignant tumors = papillomas

### Progression
- **malignant conversion to invasive carcinoma**

#### Initiation Details
- Basal compartment of epidermis
- Bulge region of hair follicle

#### Promotion Details
1. Increased DNA synthesis, inflammation.
2. Altered gene expression/enzyme activities.
3. Expansion of initiated stem cell population.
4. Production and maintenance of chronic cell proliferation.
5. Development of clonal outgrowths called papillomas.
6. Diploid lesions.

#### Progression Details
1. Additional genetic events occur stochastically.
2. Aneuploidy, LOH.
3. Dysplasia.
4. Conversion of papilloma to squamous cell carcinoma.

#### Images
- **Normal skin**
- **Hyperplastic epidermis**
- **Papilloma**
- **SCC**
Probability that Human Cutaneous Neoplastic Lesions will Progress to Invasive Carcinoma

**Mouse**

- Normal skin
- Hyperplastic epidermis
- Papilloma
- SCC

**Probabilities**

- Less than 1% (Pt w/ # < 5)
- 26% in 1 yr
- 0.5% - 3.3%

**Human**

- Normal skin + UV
- AK/SCCIS
- SCC
- Metastatic SCC

**Probabilities**

- Less than 1% (Pt w/ # ≤ 5)
- 20% (Pt w/ # ≥ 20)
- Less than 1%
The common genetic abnormalities in cSCCs

- p53 is commonly mutated in AKs and SCCs in situ indicating that p53 loss occurs prior to tumor invasion
- Aberrant activation of EGFR and Fyn.
- Activating Ras mutations in 21% of cSCCs (9% Hras, 7% Nras, 5% Kras).
# Treatment

<table>
<thead>
<tr>
<th>Drug</th>
<th>Target/Action</th>
<th>AK</th>
<th>cSCC</th>
<th>BD</th>
<th>Invasive SCC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Surgery</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5-fluorouracil*</td>
<td>Incorporates into DNA and/or RNA</td>
<td>✔️</td>
<td>✔️</td>
<td>✔️</td>
<td>?</td>
</tr>
<tr>
<td>imiquimod</td>
<td>Stimulates the immune system/produces interferon</td>
<td>✔️</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
| Picato (ingenol mebutate) | A protein kinase C inhibitor  
Induces necrosis and inflammatory response  
The first topical therapy for AK  | ✔️ |      |    |              |

- Maybe effective  ✔️ FDA-approved  ✔️ Being tested  * viable tumor remained in the deeper dermis

- Neoadjuvant use of oral retinoids (acitretin) may decrease the size of the tumor and reduce the overall tumor load in cases with multiple SCCs. However, there is currently a lack of supporting evidence from randomized studies.

- No improvement of time to recurrence or time to second primary tumors in patients with aggressive SCC treated with adjuvant 13-cis-retinoic acid plus interferon alpha for 6 months following surgery and/or radiation therapy.

- Remission rates of up to 80% have been reported for combined treatments in some studies (e.g. with 5-fluorouracil). However, the responses are mostly short lived and are followed by rapid recurrence.

- There are no targeted therapies.
Key Signaling Pathways Involved in SCC Growth

In K14-Fyn Y528F mice: lesions resembling AKs and SCCs
Akt/mTOR
MEK/ERK
STAT3

- Substrate/negative regulator of Fyn
- Inhibits KC proliferation
- Promotes differentiation

UVB → EGFR → Ras → Raf → MEK → ERK → c-Jun

Apoptosis
Senescence
Survival

Cytosol

Nucleus

p53
Bax
Bak

BAD
Bcl-2
Bcl-XL

APAF-1

ERK
Cyclin D1
p21

Cell proliferation

p53

# Hereditary Syndromes Associated with SCC

- **Xeroderma pigmentosum (XP):** a hereditary disorder of nucleotide excision repair that results in cutaneous malignancies in the first decade of life.
  - Sensitivity to sunlight
  - NMSC 150-fold

## Conditions

<table>
<thead>
<tr>
<th>Condition</th>
<th>Gene(s)</th>
<th>Clinical Testing Availability</th>
<th>Pathway</th>
</tr>
</thead>
<tbody>
<tr>
<td>Xeroderma pigmentosum</td>
<td>XPA (OMIM), XPD/ERCC3 (OMIM), XPC (OMIM), XPF/ERCC2 (OMIM), XPE/ERCC4 (OMIM), XPG/ERCC5 (OMIM)</td>
<td>No</td>
<td>Nucleotide excision repair</td>
</tr>
<tr>
<td>Xeroderma pigmentosum variant (OMIM)</td>
<td>POLH1/XPV (OMIM)</td>
<td>No</td>
<td>Error-prone polymerase</td>
</tr>
<tr>
<td>Multiple self-healing squamous epitheloma (Ferguson-Smith syndrome) (OMIM)</td>
<td>TGFB1 (OMIM)</td>
<td>No</td>
<td>Growth factor signaling</td>
</tr>
<tr>
<td>Oculocutaneous albinism (type IA [OMIM], type IB [OMIM], type II [OMIM], type III [OMIM], and type IV [OMIM])</td>
<td>TYR (OMIM), OCA2 (OMIM), SLC4A2/MATP/OCA4 (OMIM), TYRP1 (OMIM)</td>
<td>No</td>
<td>Melanin synthesis</td>
</tr>
<tr>
<td>Hermansky-Pudlak syndrome (OMIM)</td>
<td>HPS1 (OMIM), HPS3 (OMIM), HPS4 (OMIM), HPS5 (OMIM), HPS6 (OMIM), HPS7 (OMIM), HPS7/DTNB1 (OMIM), HPS8/BLOC1S3 (OMIM)</td>
<td>No</td>
<td>Melanosomal and lysosomal storage</td>
</tr>
<tr>
<td>Fanconi anemia (OMIM)</td>
<td>FANCA (OMIM), FANCB (OMIM), FANCC (OMIM), FANCD1/BRC2 (OMIM), FANCD2 (OMIM), FANCE (OMIM), FANCF (OMIM), FANCG/XRC9 (OMIM), FANCI (OMIM), FANCJ/BRIP1 (OMIM), FANCL (OMIM), FANCN (OMIM), FANCN/FALB2 (OMIM)</td>
<td>No</td>
<td>DNA repair</td>
</tr>
<tr>
<td>Dyskeratosis congenita (OMIM)</td>
<td>DKC1 (OMIM), TERC (OMIM), TINF2 (OMIM), NHP2/NOL2A (OMIM), NOP10/NOL3 (OMIM), TERT (OMIM), WRAP53 (OMIM), C16orf57 (OMIM), RTE1 (OMIM)</td>
<td>No</td>
<td>Telomere maintenance and trafficking</td>
</tr>
<tr>
<td>Rothmund-Thomson syndrome (OMIM)</td>
<td>RECQL4 (OMIM), C16orf57 (OMIM)</td>
<td>No</td>
<td>Chromosomal stability</td>
</tr>
<tr>
<td>Bloom syndrome (OMIM)</td>
<td>BLM/RECQL3 (OMIM)</td>
<td>No</td>
<td>Chromosomal stability</td>
</tr>
<tr>
<td>Werner syndrome (OMIM)</td>
<td>WRN/RECQL2 (OMIM)</td>
<td>No</td>
<td>Chromosomal stability</td>
</tr>
</tbody>
</table>

*a* For more information on genetic testing laboratories, refer to the NIH Genetic Testing Registry.

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J Clin Invest. 2011 121(1):195-211
Potential Utility of DNA Repair Enzymes

• DNA endonuclease V (T4N5), derived from a UV-resistant microbe found in marine waters/soils

• It has shown to remove CPDs

• Topical administration of liposome-encapsulated T4N5 showed 68% reduction in new AK, and 30% reduction in new BCCs in XP patients (n=30, 3 month, daily). *Lancet, 2001; J Drugs Dermatol. 2010*
Basal Cell Carcinoma (BCC)
The Hedgehog (Hh) Pathway

- Role in cell fate, growth, and differentiation;
- Critical in governing embryonic development and adult tissue homeostasis (establishing left-right body asymmetry and limb patterning, eye, brain, and central nervous system development);
- Sonic Hedgehog (SHH), Indian Hedgehog (IHH; the development of cartilage) and Desert Hedgehog (DHH; the development of male germ cells);
- Among these, SHH is the most potent and most often expressed in embryonic and adult tissues.
Hh Signaling

**INHIBITION**

**ACTIVATION**
Aberrant Hh Signaling Drives BCC Pathogenesis

Basal Cell Nevus Syndrome (BCNS) or Gorlin Syndrome

- An autosomal dominant disorder with an estimated prevalence of 1 in 57,000.
- Inherit one mutated copy of the *patched (PTCH)* gene.
- Tumors in BCNS individuals likely to arise with inactivation of the remaining PTCH allele.
- PTCH haploinsufficiency is responsible for the developmental abnormalities (e.g., multiple jaw cysts, broadening of the nasal root, rib/spine anomalies etc.)
- BCNS patients develop hundreds of BCCs.
- Other tumors: medulloblastoma, rhabdomyosarcoma
The Cellular Origin of BCC

The IFE (interfollicular epidermis) origin of SmoM2-induced BCCs in mouse-tail epidermis
Phenotypes of BCC subtypes are regulated by not only the cell of origin but also the tissue context and the level of oncogenic signaling.

From a subset of stem cells in the lower bulge and secondary hair germ compartment.

Requires high-level signaling in the interfollicular epidermis.
Ingestion of the plant alkaloid by sheep during gestation period induced cyclopia, limb defects, and tracheal stenosis.

IPI-926 (saridegib)
Vismodegib (Erivedge)
BMS-833923 (XL139)
LDE-225 (sonidegib)
NVP-LEQ506 (Smo D473H)
Vismodegib reduces the tumor burden and blocks growth of new BCCs in BCNS patients, but...
Limitations of SMO-Targeted Therapies

- The rapid development of **drug resistance** in a subpopulation of BCCs, leading to treatment failure, and **tumor relapse/recurrence**.

- Secondary **mutations in SMO** (e.g., the drug-binding pocket) and, to a lesser extent, through concurrent changes in copy number in *SUFU* and *GLI2*, leading to **impairing drug binding and/or reactivating the Hh pathway**.

- A range of **adverse effects**: loss of taste, hair loss, weight loss, gastrointestinal distress, muscle cramps, and fatigue.

- Vismodegib-resistant tumors have also proven refractory to sonidegib, a newer FDA-approved SMO inhibitor.
SMO Variants Explain Both Intrinsic and Acquired Drug Resistance

Cancer Cell. 2015 Mar 9;27(3):342-53
### Hh pathway modulators with different mechanisms of action

<table>
<thead>
<tr>
<th>Hh pathway modulator</th>
<th>Mechanism of action</th>
<th>Comment</th>
<th>Status b</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Upstream of or at SMO</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SMANT</td>
<td>Inhibits cilia accumulation, distinct binding mode on SMO</td>
<td>Weak competition with cyclosporine; active on SMO M2</td>
<td>Research</td>
</tr>
<tr>
<td>CA1 and CA2</td>
<td>Inhibit cilia biogenesis, do not bind SMO</td>
<td></td>
<td>Research</td>
</tr>
<tr>
<td>Glucocorticoids a, class I (FA and TA)</td>
<td>Induce SMO accumulation in cilia</td>
<td>Compete with cyclosporine; enhance Hh pathway activation; interfere with action of SMO inhibitors</td>
<td>Research (in clinical use for other indications)</td>
</tr>
<tr>
<td>Glucocorticoids a, class II (Bud and Cic)</td>
<td>Inhibit cilia accumulation of SMO</td>
<td>Do not compete with cyclosporine; active on resistant mutation D473H and SMO M2</td>
<td>Research (in clinical use for other indications)</td>
</tr>
<tr>
<td>Itraconazole a</td>
<td>Prevents cilia translocation of SMO</td>
<td>Does not compete with cyclosporine; active on resistant mutation D473H but not SMO M2</td>
<td>In clinical evaluation for Hh-driven cancers; phase 2 study in chemotherapy-naive metastatic castration-resistant prostate cancer: 24-week PPFS, 48% (600 mg dose, n = 29), 11.8% (200 mg dose, n = 17); median PFS, 35.9 weeks (600 mg dose) compared to 11.9 weeks (200 mg dose)</td>
</tr>
<tr>
<td>ALLO1 and ALLO2</td>
<td>Distinct binding mode on SMO</td>
<td>Active on D473H (ALLO1 and ALLO2) and SMO M2</td>
<td>Research</td>
</tr>
<tr>
<td>Compound 5</td>
<td>Binds SMO and inhibits ciliary translocation</td>
<td>Active on D473H, inhibits in vivo growth of vismodegib-resistant tumors</td>
<td>Research</td>
</tr>
<tr>
<td>Robotnikinin</td>
<td>Binds to SHH and blocks pathway activity</td>
<td>Different MOA than SMO inhibitors</td>
<td>Research</td>
</tr>
<tr>
<td>RU-SKI</td>
<td>Inhibits Hh acyltransferase</td>
<td>Interferes with SHH palmitoylation and blocks SHH signaling</td>
<td>Research</td>
</tr>
<tr>
<td>Hh-specific monoclonal antibody SE1</td>
<td>Blocks binding of Hh ligands to PTCH1</td>
<td>Used widely to demonstrate Hh dependency in tumor models</td>
<td>Research</td>
</tr>
<tr>
<td><strong>Downstream of SMO</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GANTS8 and GANT61</td>
<td>Block GLI1- and GLI2-mediated reporter activity; GANT61 interferes with DNA binding of GLI1</td>
<td></td>
<td>Research</td>
</tr>
<tr>
<td>HPI 1–4</td>
<td>Act at or downstream of SUFU; modulate GLI1 processing, activation and or trafficking</td>
<td></td>
<td>Research</td>
</tr>
<tr>
<td>Arsenics a</td>
<td>Act at level of GLI</td>
<td>Two proposed mechanisms: inhibition of Hh-induced ciliary accumulation of GLI2 or direct binding and inhibition of GLI1 independent of primary cilia</td>
<td>Research (in clinical use for other indications; <a href="http://www.fdaapproveddrugs.us/trisenox.htm">http://www.fdaapproveddrugs.us/trisenox.htm</a>)</td>
</tr>
<tr>
<td>Myristoylated aPKC peptide inhibitor (PSI)</td>
<td>Inhibits the phosphorylation and activation of GLI1 by aPKC-</td>
<td>Inhibits growth of SMO inhibitor–resistant mouse BCC lines</td>
<td>Research</td>
</tr>
<tr>
<td>Rapamycin</td>
<td>Inhibits TNF-α–induced and mTOR-S6K–mediated phosphorylation and activation of GLI1 in EAC lines</td>
<td></td>
<td>Research</td>
</tr>
</tbody>
</table>

*High dose needed to achieve efficacious plasma levels. Status in regard to Hh-driven cancers. Bud, budesonide; Cic, ciclesonide; FA, flucinolone acetonide; MOA, mechanism of action; PPFS, prostate-specific antigen progression-free survival; TA, triamcinolone acetonide.
Hh targeting alone is insufficient; additional therapeutic targets/strategies are needed.

Ptch1+/−/SKH-1 Mouse: A Model for BCNS

*Oncotarget*, 2015
Akt1 Haplodeficiency Suppresses UV-induced Growth of BCCs in Akt1\(^{+/−}\)/Ptch1\(^{+/−}\)/SKH-1 Mice

C. 

Graph showing the effect of UV irradiation on tumor volume and number per animal across different genotypes (WT/WT, Ptch1\(^{+/−}\), Akt1\(^{+/−}\)/Ptch1\(^{+/−}\)).

D. 

Bar graphs showing the average mBCC area and number per animal for Ptch1\(^{+/−}\), Akt1\(^{+/−}\)/Ptch1\(^{+/−}\) genotypes, with statistical significance indicated by p-values (p = 0.029, p = 0.007).
Perifosine (alkylphospholipid, 50 mg/kg, PO BID, twice a day, four weeks) combined with itraconazole or vismodegib (100 mg/kg PO BID, twice a day, for four weeks) to chronically UV-irradiated Ptch1+/−/SKH-1 mice.
Melanoma
- Originates in the pigment-producing **melanocytes** in the basal layer of the epidermis.

- Melanomas often resemble moles; some develop from moles. The majority of melanomas are black or brown, but they can also be skin-colored, pink, red, purple, blue or white (not uniform in color).
ABCDE: Rule for the Early Detection of Melanoma

- **Asymmetrical**
- **Borders are uneven**
- **Multiple Colors/shades**
- **Diameter > ¼ inch**

**Evolution:** changing in size, shape and color
Exposure to extracellular ligands

↓

Activation of RTKs and the recruitment of adaptor protein complexes (GRB2-SOS) to the plasma membrane

↓

The exchange of GDP for GTP by RAS, resulting in RAS activation

↓

RAF activation (conformational changes, phosphorylation and dimerization)

↓

MEK activation

↓

ERK activation

- Cell cycle progression and survival.

- ERK directly phosphorylates and inactivates upstream signaling intermediates (direct negative feedback, solid lines).

- ERK regulates SPRY and DUSPs, inhibitors (dotted lines).

- SPRY impedes signaling by disrupting the GRB2-SOS interaction; DUSPs are ERK-specific phosphatases.
BRAF is the Single Most Commonly Mutated Gene in Melanoma

- ARAF, BRAF, and CRAF serine/threonine protein kinase; BRAF has the highest basal kinase activity.

- Activating mutations in BRAF are present in approximately 40 to 60% of advanced melanomas.

- In 80 to 90% of cases, this activating mutation consists of V600E. BRAF^{V600E} can gain 500-fold increased activation.

- BRAF mutations are usually found in tumors with no driver mutations in NRAS, KIT, and other genes.

- BRAF^{V600E} is prevalent in benign nevi and believed to play a central role in the formation of early and benign melanocytic growth.
Hyperactivated ERK signaling results in excessive cell proliferation and survival, and evasion of apoptosis.

In this state, BRAF V600E signals as a functional monomer.

Constitutive activation is independent of extracellular factors.

Not responsive to normal regulatory signals.
Clinical Efficacy of the BRAF Inhibitor PLX4032 (Vemurafenib)

Clinical endpoint: progression-free survival (%)

The recommended phase 2 dose: 960 mg twice daily

Chapman et al, NEJM 2011; Flaherty et al, NEJM 2010
ODDP 2014, Amsterdam (Alain van Gool)
Acquired Resistance to PLX4032 (Vemurafenib)

Before Rx

Tumors refractory to several therapeutic regimens

PLX4032-15 wks

Impressive initial effects

PLX4032-23 wks

Relapse

Drug resistance
Tumor recurrence

JCO 2011;29:3085-3096
Major mechanisms of adaptive response or acquired resistance to RAF and MEK inhibitors

- Upregulation of receptor tyrosine kinase (RTK)
- RAS mutation
- Neurofibromin 1 (NF1) loss
- BRAF(V600E) splicing variants that constitutively dimerize
- BRAF amplifications
- CRAF overexpression
- MEK mutations
- TPL2 (mitogen-activated protein kinase kinase kinase 8) overexpression

Increased ERK signaling
Resistance to RAF, MEK and ERK inhibitors
Additional Molecular Changes and Melanoma Progression

- The full oncogenic potential of BRAF appears to be dictated by the presence or absence of other genetic constraints (e.g., inactivation of p16INK4a/p14ARF, p53, or PTEN; activation of PIK3CA).

- Familial atypical mole melanoma (FAMM) syndrome, which is associated with CDKN2A mutations, vastly increases the risk of melanoma.
A Stepwise decrease in p16

Biallelic inactivation of CDKN2A, emerging exclusively in invasive melanomas.

BRAF V600E mutations are present exclusively in benign lesions.
Expression of mutationally activated PIK3CA$^{H1047R}$ in the melanocytes is sufficient to drive BRAF$^{V600E}$-initiated melanomagenesis

- Pharmacological inhibition of PI3Ks prevents the growth of BRAF$^{V600E}$/PTEN$^{Null}$ melanomas in vivo.
A Model for Progression of Melanomas Developing on Sun-Exposed Sites

<table>
<thead>
<tr>
<th>Histology</th>
<th>Benign Lesion</th>
<th>Intermediate Lesion</th>
<th>Melanoma In Situ</th>
<th>Invasive Melanoma</th>
<th>Metastasis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Evolutionary Model</td>
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<tr>
<td>Point Mutations</td>
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<tr>
<td>Copy-Number Alterations</td>
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<tr>
<td>Mutation Signature</td>
<td>Ultraviolet radiation</td>
<td></td>
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<tr>
<td>Driver Genes</td>
<td>BRAF V600E</td>
<td>TERT</td>
<td>NRAS + TERT</td>
<td>BRAF V600K + TERT</td>
<td>CDKN2A genes encoding SWI/SNF subunits</td>
</tr>
</tbody>
</table>

Telomerase reverse transcriptase
Targeting Melanoma Signaling Networks

The growth-promoting Ras signaling network
- Vemurafenib (BRAF<sup>V600E</sup>)
- Dabrafenib (BRAF<sup>V600E</sup>)
- Encorafenib (BRAF<sup>V600E</sup>)
- Trametinib (MEK1/2)
- Cobimetinib (Mek)
- Binimetinib (Mek)

The tumor-constraining CDKN2A network

PI3K-AKT cascade: promoter of melanoma progression

Malignant melanoma is frequently driven by mutational activation of BRAF accompanied by silencing of the PTEN tumor suppressor.
Immunotherapy: Predicting Antitumor Response

- **PD-L1 IHC expression** is an important predictive biomarker and currently the only biomarker approved for clinical use — discordance between response rates; a considerable proportion of patients with PD-L1-negative tumors do respond

- **Higher tumor mutational burden** increases in neoantigens, which in turn could result in the induction of both a CD4+ and CD8+ T-cell response — insufficient to prevent the formation and progression of melanomas (ineffective epitope presentation, reduced expression of potent antigens, expression of additional immunomodulatory molecules by tumor cells?)

- More **CD8 infiltration**, as well as PD-1 and PD-L1 expression at the invasive tumor margin?

- **PD-L1 and PD-L2 gene copy number gains**?

- **Microsatellite instability (MSI)?**: Deficiencies in DNA mismatch repair (MMR) lead to the accumulation of cancer genomic errors, resulting in microsatellite fragments. MSI-high status has prognostic significance in colorectal cancer in relation to adjuvant chemotherapy in addition to predictive significance for anti-PD-1-directed therapy.

- Multiple parameter combinations?
## Melanoma Therapy

<table>
<thead>
<tr>
<th>Therapy/study</th>
<th>ORR (%)</th>
<th>PFS</th>
<th>OS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Median (mo)</td>
<td>1y (%)</td>
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<tr>
<td>Checkpoint inhibitors</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Ipilimumab (CTLA-4)</td>
<td>11–19</td>
<td>2.8–3.3</td>
<td>18–19</td>
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<tr>
<td>Pembrolizumab (PD-1)</td>
<td>36–37</td>
<td>8.3</td>
<td>38–39</td>
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<tr>
<td>Nivolumab (PD-1)</td>
<td>40–44</td>
<td>5.4–6.9</td>
<td>42–44</td>
</tr>
<tr>
<td>Ipilimumab + nivolumab</td>
<td>57–58</td>
<td>11.5</td>
<td>49–53</td>
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<tr>
<td>Targeted therapy</td>
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<td></td>
<td></td>
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<tr>
<td>Vemurafenib</td>
<td>50–51</td>
<td>7.2–7.3</td>
<td>NR</td>
</tr>
<tr>
<td>Dabrafenib</td>
<td>53</td>
<td>8.8</td>
<td>NR</td>
</tr>
<tr>
<td>Encorafenib</td>
<td>51</td>
<td>9.6</td>
<td>NR</td>
</tr>
<tr>
<td>Vemurafenib + cobimetinib (V600E/MEK)</td>
<td>70</td>
<td>12.3</td>
<td>52</td>
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<tr>
<td>Dabrafenib + trametinib (V600E/MEK)</td>
<td>64–69</td>
<td>11–12</td>
<td>NR</td>
</tr>
<tr>
<td>Encorafenib + binimetinib (V600E/MEK)</td>
<td>63</td>
<td>14.9</td>
<td>NR</td>
</tr>
</tbody>
</table>

NR not reported, ORR overall response rate, OS overall survival, PFS progression-free survival

### Immunotherapy
- Highest chance of durable response
- Response can be ongoing even after therapy has been stopped
- Time to response might be longer
- Substantial fraction of patients do not respond

### Targeted therapy
- High response rate
- Fast and deep tumor response
- Therapy not hampered by other immunosuppressive agents
- Majority of patients will develop resistance
A relatively low mutational rate; modest efficacy with MEKi at the cost of significant toxicity

Non-cutaneous Melanoma

Uveal
GNAQ 32%
G11 45%

Face/Scalp
NRAS 15%
BRAF 28%

Trunk/Legs
NRAS 18%
BRAF 57%

ACRAL
C-KIT 5-10%
NRAS 25%
BRAF 10%

Mucosal
NRAS 15%
C-KIT 10-20 %

PD-1 inhibition as first-line therapy

GNAQ, guanine nucleotide binding protein, q polypeptide
G11, guanine nucleotide-binding protein subunit alpha 11
How resistance to targeted therapies develops and how to overcome potential crosstalk with other signaling pathways

Strategies leading to optimized patient selection and enhanced synergy of combination regimens (e.g., mutational profiles or mutational loads)

Validated predictive and prognostic biomarkers

Antibodies targeting additional immune-checkpoint molecules
UV Exposure is a Major Cause of Skin Cancer—and the Most Preventable

Sun Protection Strategies

- Wear Protective Clothing
- Wear a Hat and Sunglasses
- Seek Shade
- Avoid Times of Peak Sunlight
- Use Sunscreen
- Avoid Indoor Tanning and Sunbathing

<table>
<thead>
<tr>
<th>FDA Monograph Sunscreen Ingredients</th>
<th>Amount of Ray Protection</th>
<th>Chemical (C) or Physical (P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aminobenzoic acid (PABA)</td>
<td>●</td>
<td>C</td>
</tr>
<tr>
<td>Avobenzone</td>
<td>●</td>
<td>C</td>
</tr>
<tr>
<td>Cinoxate</td>
<td>☐</td>
<td>C</td>
</tr>
<tr>
<td>Dioxobenzone</td>
<td>☐</td>
<td>C</td>
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<tr>
<td>Ecamule</td>
<td>☐</td>
<td>C</td>
</tr>
<tr>
<td>Homosalate</td>
<td>☐</td>
<td>C</td>
</tr>
<tr>
<td>Methyl anthranilate</td>
<td>☐</td>
<td>C</td>
</tr>
<tr>
<td>Octocrylene</td>
<td>☐</td>
<td>C</td>
</tr>
<tr>
<td>Octyl methoxycinnamate</td>
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<td>C</td>
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<tr>
<td>Octyl salicylate</td>
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<td>C</td>
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<tr>
<td>Oxizobenzene</td>
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<td>Padimate O</td>
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<tr>
<td>Phenylezinimidazole</td>
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<td>Sulisobenzone</td>
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<tr>
<td>Titanium dioxide</td>
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<tr>
<td>Trolamine salicylate</td>
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<td>C</td>
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<tr>
<td>Zinc Oxide</td>
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<td>P</td>
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</tbody>
</table>

Protection Level: ● = extensive ○ = considerable ☐ = limited ○ = minimal

For the most up-to-date information on approved sunscreen ingredients, visit the FDA website at <http://fda.gov>. 