Skin Cancer: Biology & Therapy

Arianna L. Kim, Ph.D.

Department of Dermatology
Outline

- Skin structure
- Skin cancer (SCC, BCC, melanoma)
  - Skin cancer facts (incidence, death)
  - Causes
  - Prevention/Treatments
- Mechanisms (SCC, BCC, melanoma)
  - SCC
  - BCC
  - Melanoma
- Key issues in skin cancer research and therapy
Skin Structure

- Epidermis
- Dermis
- Hypodermis (superficial fascia)
- Hair root
- Hair follicle
- Eccrine sweat gland
- Hair follicle receptor (root hair plexus)
- Hair shaft
- Pore
- Sebaceous (oil) gland

Copyright 2004, Pearson Education, Inc., publishing as Benjamin Cummings
Human skin structure

- Epidermis
  - keratinocytes, melanocytes

- Dermis
  - dermal fibroblasts
  - blood vessels
  - nerves

- Dermal adipose tissue
  - intradermal adipocytes
  - blood vessels

Sebaceous glands

Endbulb: contains dermal papilla

Hair fiber

Hair follicle

Courtesy of Higgins, C
Normal Skin

- Stratum Corneum
- Stratum Lucidum
- Stratum Granulosum
- Stratum Spinosum
- Stratum Basale
- Dermal-Epidermal Junction
- Papillary Dermis
- Reticular Dermis

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Epidermal Cells and Layers of the Epidermis

- **Stratum Basale:**
  - Deepest epidermal layer firmly attached to the basal lamina and dermis via hemidesmosomes.
  - Consists of a single row of the youngest keratinocytes along with projections of melanocytes keratinocytes, and merkel cells.
  - This is the only layer of the epidermis where the cells are dividing.
  - As new cells are made in the S. Basale, the older cells get pushed up and become the next layer (S. Spinosum)
Cell types of the Epidermis

**Keratinocytes (90%)**
produce keratin, a waxy protein substance only found in the epidermis. It makes up the nails, hair, and is also in each superficial skin cell.

**Melanocytes (8%)**
produces melanin pigment

**Langerhan cells**
- from bone marrow/provides immunity
- dendritic cells (antigen-presenting cells)

**Merkel cells**
- located in basal layer, with short processes
- chemical synapse: between Merkel’s cell and afferent N
- function: not very clear, may be sensory epithelial cell

Langerhans cell histiocyteosis (benign)  
Langerhans cell sarcoma  
Merkel cell carcinoma
Skin Cancer

Three major types:

Basal Cell Carcinoma (BCC)
Squamous Cell Carcinoma (SCC)

melanoma
Non-melanoma Skin Cancer

- **The most common form of cancer in the United States.** More than 3.5 million skin cancers in over two million people are diagnosed annually.

- Each year there are more new cases of skin cancer than the combined incidence of cancers of the breast, prostate, lung and colon.

- Treatment of skin cancers increased by nearly 77% between 1992 and 2006.

- Over the past three decades, more people have had skin cancer than all other cancers combined.

- One in five Americans will develop skin cancer in the course of a lifetime.
Melanoma

- From 1970 to 2009, the incidence of melanoma increased by 800% among young women and 400% among young men.

- Melanoma is the most common form of cancer for young adults 25-29 years old and the second most common form of cancer for young people 15-29 years old.

- Survivors of melanoma are about nine times as likely as the general population to develop a new melanoma.

- Melanoma is one of only three cancers with an increasing mortality rate for men, along with liver cancer and esophageal cancer.
• Of the seven most common cancers in the US, melanoma is the only one whose incidence is increasing.

• Between 2000 and 2009, incidence climbed 1.9% annually.

*Age-adjusted to the 2000 US standard population and adjusted for delays in reporting.

*Age-adjusted to the 2000 US standard population and adjusted for delays in reporting.

Source: Surveillance, Epidemiology, and End Results Program, Delay-adjusted Incidence database:
Estimated New Cancer Cases* in the US in 2013

<table>
<thead>
<tr>
<th></th>
<th>Men</th>
<th>Women</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>854,790</td>
<td>805,500</td>
</tr>
<tr>
<td>Prostate</td>
<td>28%</td>
<td></td>
</tr>
<tr>
<td>Lung &amp; bronchus</td>
<td>14%</td>
<td></td>
</tr>
<tr>
<td>Colon &amp; rectum</td>
<td>9%</td>
<td></td>
</tr>
<tr>
<td>Urinary bladder</td>
<td>6%</td>
<td></td>
</tr>
<tr>
<td><strong>Melanoma of skin</strong></td>
<td>5%</td>
<td>4%</td>
</tr>
<tr>
<td>Kidney &amp; renal pelvis</td>
<td>5%</td>
<td>4%</td>
</tr>
<tr>
<td>Non-Hodgkin lymphoma</td>
<td>4%</td>
<td>3%</td>
</tr>
<tr>
<td>Oral cavity</td>
<td>3%</td>
<td></td>
</tr>
<tr>
<td>Leukemia</td>
<td>3%</td>
<td></td>
</tr>
<tr>
<td>Pancreas</td>
<td>3%</td>
<td></td>
</tr>
<tr>
<td>All Other Sites</td>
<td>20%</td>
<td></td>
</tr>
<tr>
<td>29% Breast</td>
<td>14% Lung &amp; bronchus</td>
<td></td>
</tr>
<tr>
<td>9% Colon &amp; rectum</td>
<td>6% Uterine corpus</td>
<td></td>
</tr>
<tr>
<td>6% Thyroid</td>
<td>4% Non-Hodgkin lymphoma</td>
<td></td>
</tr>
<tr>
<td>3% Kidney &amp; renal pelvis</td>
<td>3% Pancreas</td>
<td></td>
</tr>
<tr>
<td>3% Ovary</td>
<td>19% All Other Sites</td>
<td></td>
</tr>
</tbody>
</table>

*Excludes basal cell and squamous cell skin cancers and in situ carcinoma except urinary bladder.

Melanoma is the 5th most common cancer for males and 7th most common for females. Five percent of all cancers in men are melanomas; four percent of all cancers in women are melanomas.
The Lifetime Probability of Developing Cancer for Men and Women, 2007-2009*

<table>
<thead>
<tr>
<th>Site</th>
<th>Risk</th>
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</thead>
<tbody>
<tr>
<td>All sites†</td>
<td>1 in 2</td>
</tr>
<tr>
<td>Prostate</td>
<td>1 in 6</td>
</tr>
<tr>
<td>Lung and bronchus</td>
<td>1 in 13</td>
</tr>
<tr>
<td>Colon and rectum</td>
<td>1 in 19</td>
</tr>
<tr>
<td>Urinary bladder‡</td>
<td>1 in 26</td>
</tr>
<tr>
<td><strong>Melanoma§</strong></td>
<td>1 in 35</td>
</tr>
<tr>
<td>Non-Hodgkin lymphoma</td>
<td>1 in 43</td>
</tr>
<tr>
<td>Kidney</td>
<td>1 in 49</td>
</tr>
<tr>
<td>Leukemia</td>
<td>1 in 63</td>
</tr>
<tr>
<td>Oral Cavity</td>
<td>1 in 66</td>
</tr>
<tr>
<td>Stomach</td>
<td>1 in 92</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Site</th>
<th>Risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>All sites†</td>
<td>1 in 3</td>
</tr>
<tr>
<td>Breast</td>
<td>1 in 8</td>
</tr>
<tr>
<td>Lung &amp; bronchus</td>
<td>1 in 16</td>
</tr>
<tr>
<td>Colon &amp; rectum</td>
<td>1 in 21</td>
</tr>
<tr>
<td>Uterine corpus</td>
<td>1 in 38</td>
</tr>
<tr>
<td>Non-Hodgkin lymphoma</td>
<td>1 in 52</td>
</tr>
<tr>
<td>Urinary bladder‡</td>
<td>1 in 87</td>
</tr>
<tr>
<td><strong>Melanoma§</strong></td>
<td>1 in 54</td>
</tr>
<tr>
<td>Ovary</td>
<td>1 in 72</td>
</tr>
<tr>
<td>Pancreas</td>
<td>1 in 69</td>
</tr>
<tr>
<td>Uterine cervix</td>
<td>1 in 147</td>
</tr>
</tbody>
</table>

* For those free of cancer at beginning of age interval.
† All sites exclude basal and squamous cell skin cancers and in situ cancers except urinary bladder.
‡ Includes invasive and in situ cancer cases
§ Statistic for white men.
### Trends in Five-year Relative Cancer Survival Rates (%), 1975-2008

<table>
<thead>
<tr>
<th></th>
<th></th>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>All sites</td>
<td>49</td>
<td>56</td>
<td>68</td>
</tr>
<tr>
<td>Breast (female)</td>
<td>75</td>
<td>84</td>
<td>90</td>
</tr>
<tr>
<td>Colon</td>
<td>51</td>
<td>61</td>
<td>65</td>
</tr>
<tr>
<td>Leukemia</td>
<td>34</td>
<td>43</td>
<td>58</td>
</tr>
<tr>
<td>Lung &amp; bronchus</td>
<td>12</td>
<td>13</td>
<td>17</td>
</tr>
<tr>
<td>Melanoma</td>
<td>82</td>
<td>88</td>
<td>93</td>
</tr>
<tr>
<td>Non-Hodgkin lymphoma</td>
<td>47</td>
<td>51</td>
<td>71</td>
</tr>
<tr>
<td>Ovary</td>
<td>36</td>
<td>38</td>
<td>43</td>
</tr>
<tr>
<td>Pancreas</td>
<td>2</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td>Prostate</td>
<td>68</td>
<td>83</td>
<td>100</td>
</tr>
<tr>
<td>Rectum</td>
<td>48</td>
<td>58</td>
<td>68</td>
</tr>
<tr>
<td>Urinary bladder</td>
<td>73</td>
<td>79</td>
<td>80</td>
</tr>
</tbody>
</table>


Estimated number of new cases

Melanoma accounts for less than 5% of skin cancer cases, but it has a vast majority of skin cancer deaths.
Causes and Risk Factors

- **Ultraviolet (UV) radiation** (sun, tanning bed)
- Pigmentary characteristics
- 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.
  - In many parts of the world, wells providing drinking water are contaminated by high levels of arsenic in the ground water. 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.
- Immunosuppression
  - Among solid-organ transplant recipients, 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 of non-melanoma skin cancer
- **Other radiation exposure**
  - Exposure to therapeutic radiation (e.g., psoralen and UVA (PUVA)) > a three-fold to six-fold increase in SCC.

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

- **Nevi**
  - Patients with **multiple nevi** demonstrate increased risk of melanoma. While there is evidence that both the presence of multiple nevi and the presence of multiple clinically atypical nevi are associated with an increased risk of melanoma, **most studies demonstrate a stronger risk of melanoma with the presence of atypical nevi.**
UV radiation is the primary risk factor

- About 90% of non-melanoma skin cancers are associated with exposure to UV radiation from the sun.

- About 86% of melanomas can be attributed to exposure to UV radiation from the sun.
  - The vast majority of mutations found in melanoma are caused by UV radiation.
  - A person’s risk for melanoma doubles if he or she has had more than five sunburns.

- Regular daily use of an SPF 15 or higher sunscreen reduces the risk of developing SCC by 40% and the risk of developing melanoma by 50%.
Specific patterns of sun exposure appear to lead to different types of skin cancer among susceptible individuals

- Intense intermittent recreational sun exposure has been associated with melanoma and BCC.
- Chronic occupational sun exposure has been associated with SCC.
Electromagnetic spectrum of visible and UV radiation and biologic effects on the skin

- Visible Light
- UVA
- UVB
- UVC

Solar Radiation

Terrestrial Radiation

Stratosphere

Epidermis

Dermis

Reactive Oxygen Species

Direct Energy Absorption by DNA

DNA damage

- Strand breaks
- Abasic sites
- Oxidative damage
- Base Modifications

- Cyclopyrimidine dimers
- 6-4 photoproducts

Mutations, Cancer

Though highly reactive with DNA, UVC is absorbed by atmospheric ozone and is not a major component of terrestrial UV radiation in most places on Earth.
Pyrimidine dimers result from ultraviolet light

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

These lesions are repaired by the excision repair pathway, but when unrepaiired can form the classic “UVB signature” mutations: $C \rightarrow T$ or $CC \rightarrow TT$, which are found in skin cancers.

UVA is also mutagenic, but its genetic effects have been mainly attributed 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.

UVB also induces 8-oxo-7,8-dihydroguanine.
Cyclobutane pyrimidine dimers (CPDs) are predominant DNA lesions in whole human skin exposed to UVA radiation. Proc Natl Acad Sci U S A. 2006 Sep 12;103(37):13765-70.

- T<>T was found to be the major lesion produced after both UVA and UVB irradiations in the DNA of human skin.
- T<>T retained in large amount within the DNA of human skin 48 h after the end of the irradiations.

UVA caused more intense staining of CPDs in the lower epidermis, whereas UVB led to more intense upper epidermal staining.
SCC

- 250,000 cases are diagnosed each year, resulting in approximately 2,500 deaths.
- Arise from the epidermis and have an initial progression stage in which proliferation of the neoplastic cells is confined to the epidermis.
- Molecular analyses of solar keratosis, a prototype of early SCC in situ, show that **dysfunction of p53** plays a critical role.
- There seems to be potential precursor cells to be present in in situ lesions.
- These precursor cells may be **defective in repair response to DNA damage**, and would have proliferative/survival advantages over their normal neighboring counterparts in the presence of growth factor stimulation or genotoxic events, such as UV.
SCCs
Probability that human cutaneous neoplastic lesions will progress to invasive carcinoma

<table>
<thead>
<tr>
<th>Model</th>
<th>Strengths</th>
<th>Weaknesses</th>
</tr>
</thead>
<tbody>
<tr>
<td>2D skin culture</td>
<td>Ease of use and genetic manipulation</td>
<td>Lack of features of 3D tissues</td>
</tr>
<tr>
<td></td>
<td></td>
<td>No immune regulation</td>
</tr>
<tr>
<td></td>
<td></td>
<td>No circulation</td>
</tr>
<tr>
<td>Organotypic culture</td>
<td>Direct genetic manipulation with lentiviruses</td>
<td>No immune regulation</td>
</tr>
<tr>
<td></td>
<td>Architecturally faithful native 3D matrix and stroma</td>
<td>No circulation, diffusion limited</td>
</tr>
<tr>
<td></td>
<td>Replication of cell-ECM interaction and invasion</td>
<td></td>
</tr>
<tr>
<td>xenograft</td>
<td>Only method for studying genetically engineered functional human tissue in 3D in vivo</td>
<td>No immune regulation</td>
</tr>
<tr>
<td></td>
<td>Other strengths the same as organotypic culture</td>
<td>Murine host biology</td>
</tr>
<tr>
<td>Tg mouse models</td>
<td>Direct genetic engineering ability to analyze multiple genes</td>
<td>Murine skin differs from human skin</td>
</tr>
<tr>
<td>Explant human skin culture</td>
<td>Complete human skin</td>
<td>Can be used for only short time periods</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Uniform genetic manipulation difficult</td>
</tr>
</tbody>
</table>
During initiation, topical application of a sub-carcinogenic dose of a mutagenic agent induces mutations in target genes in keratinocytes. Repeated topical application of a promoting agent begins 2 weeks after initiation and continues for the duration of the study. Papillomas begin to arise after 6–12 weeks of promotion and a fraction begin to convert to SCC after 20 weeks.

**Nature Protocols 4, 1350 - 1362 (2009)**
**A clinical, histologic, and molecular comparison of AKs, cSCC, and metastatic cSCC**

<table>
<thead>
<tr>
<th>Normal skin</th>
<th>AK</th>
<th>cSCC</th>
<th>Metastatic cSCC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical description</td>
<td>Scaly skin colored/pink macule or papule</td>
<td>Persistent firm or scaly papule or red nodule which may spontaneously bleed</td>
<td>Multiple nodular lesions in skin or internal organs</td>
</tr>
<tr>
<td>Histopathology</td>
<td>Enlarged, atypical keratinocytes confined to the epidermis with parakeratotic scale</td>
<td>Enlarged, atypical keratinocytes invading the dermis</td>
<td>Enlarged, atypical keratinocytes in the dermis, lymph nodes, or internal organs, typically with no epidermal connection</td>
</tr>
<tr>
<td>Histological description</td>
<td>Well-defined stratum basalis, spinosum, and granulosum with orthokeratotic scale</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Increased signaling** (activation, overexpression, or amplification)
- AK: ras, Fyn/SFKs, bcl-2
- cSCC: EGFR, Myc, PI3K/Akt, p16 LOH
- Metastatic cSCC: EGFR, p16 LOH

**Decreased signaling** (deactivation, transcriptional or translational repression, or gene deletion)
- AK: p53, Srcasrm
- cSCC: p53, Srcasrm, Notch (p53), PKC δ, E-cadherin
- Metastatic cSCC: E-cadherin, P-cadherin

**Genomic changes**
- AK: Genomic instability with few chromosomal alterations
- cSCC: Increased genomic instability resulting in chromosomal translocations, isochromosomes, gene deletions, and amplifications
- Metastatic cSCC: In addition to cSCC alterations: VEGF (ras), MMP2, MMP7, MMP12 (ras)
Hereditary syndromes associated with SCC

- Xeroderma pigmentosum (XP) is a hereditary disorder of nucleotide excision repair that results in cutaneous malignancies in the first decade of life.
  - Affected individuals have an increased sensitivity to sunlight, resulting in a markedly increased risk of SCCs, BCCs, and melanomas.
  - One report found that NMSC was increased 150-fold in individuals with XP.
  - For those younger than 20 years, the prevalence was almost 5,000 times what would be expected in the general population.

<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 variant (OMIM)</td>
<td>POLH2/PXP (OMIM)</td>
<td>No</td>
<td>Error-prone polymerase</td>
</tr>
<tr>
<td>Xeroderma pigmentosum (OMIM)</td>
<td>XPA (OMIM), XPB/RCC3 (OMIM), XPC (OMIM), XPD/RCC2 (OMIM), XPF/ERCC4 (OMIM), XPG/ERCC5 (OMIM)</td>
<td>No</td>
<td>Nucleotide excision repair</td>
</tr>
<tr>
<td>Multiple self-healing squamous epithelioma (Ferguson-Smith syndrome) (OMIM)</td>
<td>TGFBR1 (OMIM)</td>
<td>No</td>
<td>Growth factor signaling</td>
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<td>Ciclopirogus albinism (type I) (OMIM), type II (OMIM), type III (OMIM), type IV (OMIM)</td>
<td>TYR (OMIM), OCA2 (OMIM), MATP/POCA4 (OMIM), TYRP1 (OMIM)</td>
<td>TYR, OCA2, TYRP1</td>
<td>Melanin synthesis</td>
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<tr>
<td>Hermansky-Pudlak syndrome (OMIM)</td>
<td>HPS1 (OMIM), HPS3 (OMIM), HPS4 (OMIM), HPS5 (OMIM), HPS7 (OMIM), HPS7/TDNBP1 (OMIM), HPS2/BLOC133 (OMIM)</td>
<td>HPS1, HPS3, HPS4, HPS7</td>
<td>Melanosomal and lysosomal storage</td>
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<tr>
<td>Hermansky-Pudlak syndrome, Type 2 (OMIM)</td>
<td>AP3B1 (OMIM)</td>
<td>No</td>
<td>Melanosomal and lysosomal storage</td>
</tr>
<tr>
<td>Chediak-Higashi syndrome (OMIM)</td>
<td>LYST (OMIM)</td>
<td>LYST</td>
<td>Lysosomal transport regulation</td>
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<tr>
<td>Giegiella syndrome (type 1 (OMIM), type 2 (OMIM), and type 3 (OMIM))</td>
<td>MYO5A (OMIM), RARP2TA (OMIM), MLPH (OMIM)</td>
<td>RARP2TA</td>
<td>Pigment granule transport</td>
</tr>
<tr>
<td>Ehlers-Danlos Disease (OMIM)</td>
<td>MYO5A (OMIM)</td>
<td>No</td>
<td>Pigment granule transport</td>
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<tr>
<td>Dystrophic epidermolysis bullosa (OMIM)</td>
<td>COL7A1 (OMIM)</td>
<td>COL7A1</td>
<td>Collagen anchor of basement membrane to dermis</td>
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<tr>
<td>Junctional epidermolysis bullosa (OMIM)</td>
<td>LAM3A (OMIM), LAMB3 (OMIM), LAMC2 (OMIM), COL7A1 (OMIM)</td>
<td>LAM3A, LAMB3, LAMC2, COL7A1</td>
<td>Connective tissue</td>
</tr>
<tr>
<td>Epidermolyticplasia verruciformis (OMIM)</td>
<td>EVER1 (OMIM), EVER2 (OMIM)</td>
<td>No</td>
<td>Signal transduction in endoplasmic reticulum</td>
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<td>Fanconi anemia (OMIM)</td>
<td>FANCA (OMIM), FANCC (OMIM), FANCG (OMIM), FANCI (OMIM), FANCJ (OMIM), FANCJ/BRIP1 (OMIM), FANCL (OMIM), FANCM (OMIM), FANCN/PALB2 (OMIM)</td>
<td>Chromosomal breakage testing: BRIP1, FANCA, FANCC, FANCJ, FANCF, PALB2</td>
<td>DNA repair</td>
</tr>
<tr>
<td>Dyskeratosis congenita (OMIM)</td>
<td>DKC1 (OMIM), TERC (OMIM), TINF2 (OMIM), NHP2/NDPLA2 (OMIM), NCP101/NCL13 (OMIM), TERT (OMIM), WRAP53 (OMIM), C16orf7 (OMIM), C16orf77 (OMIM)</td>
<td>DKC1, TERC, TINF2, NHP2, NCP101, TERT</td>
<td>Telomere maintenance and trafficking</td>
</tr>
<tr>
<td>Rothmund-Thomson syndrome (OMIM)</td>
<td>RECQL4 (OMIM), C16orf7 (OMIM)</td>
<td>RECQL4</td>
<td>Chromosomal stability</td>
</tr>
<tr>
<td>Bloom syndrome (OMIM)</td>
<td>BLM/RECQL3 (OMIM)</td>
<td>Sister chromatic exchange, BLM</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>

*For more information on genetic testing laboratories, refer to the NIH Genetic Testing Registry.
Knockdown of XPC results in epithelial hyperplasia

5th day
shCtrl shXPC1

15th day
shCtrl shXPC1

XPC-KC (keratinocytes from XPC patients)
XPC knockdown induces SCC formation

(shXPC+NOX1)

(shXPC)

(shXPC1+NOX1)

(shXPC+shAKT1)

XPC knockdown induces SCC formation
TREATMENT

The choice of treatment is based on the type, size, location, and depth of penetration of the tumor, as well as the patient's age and general health.

<table>
<thead>
<tr>
<th>Treatment Method</th>
<th>Description</th>
<th>Benefits</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mohs Micrographic Surgery</td>
<td>The physician removes the tumor with a very thin layer of tissue around it. The layer is immediately checked under a microscope thoroughly. If tumor is still present in the depths or peripheries of this surrounding tissue, the procedure is repeated until the last layer examined under the microscope is tumor-free. After removal of the skin cancer, the wound may be allowed to heal naturally or be reconstructed using plastic surgery methods.</td>
<td>Saves the greatest amount of healthy tissue, appears to reduce the rate of local recurrence, and has the highest overall cure rate (~94-99 %).</td>
</tr>
<tr>
<td>Curettage and Electrodesiccation (Electrosurgery)</td>
<td>The growth is scraped off with a curette, and burning heat produced by an electrocautery needle destroys residual tumor and controls bleeding.</td>
<td></td>
</tr>
<tr>
<td>Cryosurgery</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Photodynamic Therapy (PDT)</td>
<td>Both FDA-approved for treatment of actinic keratoses and are also being tested for the treatment of some superficial SCCs. Some trials have shown that imiquimod may be effective with certain invasive SCCs, but it is not yet FDA-approved for this purpose. Imiquimod stimulates the immune system to produce interferon, a chemical that attacks cancerous and precancerous cells.</td>
<td></td>
</tr>
</tbody>
</table>
**PREVENTION**

General sun-safety measures: sun avoidance at peak hours, use of protective clothing/sunscreen, and close clinical monitoring of the skin.

Oral isotretinoin (13-cis retinoic acid; given as 2 mg/kg/day) has been used as chemoprevention in XP patients with promising results (NMSC incidence reduced by 63%).
Potential pathways that may be targeted by small molecules to treat AKs and cSCCs

![Diagram showing potential pathways for AKs and cSCCs treatment](image-url)
The most common type of skin cancer, accounts for about 90% of the skin cancers.

Almost never metastasizes or crosses the basement membrane, so is almost never fatal.

It is the most easily cured: surgical removal, no chemotherapy or radiation usually needed.

Extensive morbidity through local invasion and tissue destruction.

Recurrence is common.
Major subtypes of human BCC

A. Nodular BCC
B. Nodular BCC histology
C. Superficial BCC
D. Superficial BCC histology
E. Sclerosing BCC
F. Sclerosing BCC histology
## TREATMENT

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mohs Micrographic Surgery using local anesthesia</td>
<td></td>
</tr>
<tr>
<td>Excisional Surgery</td>
<td></td>
</tr>
<tr>
<td>Curettage and Electrodesiccation</td>
<td>The growth is scraped off with a curette, and burning heat produced by an electrocautery needle destroys residual tumor and controls bleeding.</td>
</tr>
<tr>
<td>Radiation (x-ray)</td>
<td></td>
</tr>
<tr>
<td>Cryosurgery</td>
<td></td>
</tr>
<tr>
<td>Photodynamic Therapy (PDT)</td>
<td>PDT can be useful when patients have multiple BCCs. A photosensitizing agent such as Topical 5-aminolevulinic acid (5-ALA) is applied to the tumors. It is taken up by the abnormal cells. The next day, the medicated areas are activated by a strong light. This treatment selectively destroys BCCs while causing minimal damage to surrounding normal tissue. PDT is FDA approved for treatment of superficial and nodular BCCs. Cure rates can vary considerably, ranging from 70 to 90 percent. Patients become photosensitive for 48 hours after the treatment and must stay out of the sun.</td>
</tr>
<tr>
<td>Topical Medications:</td>
<td></td>
</tr>
<tr>
<td>Imiquimod (5% cream)</td>
<td>Imiquimod is FDA-approved only for superficial BCCs, with cure rates generally between 80 and 90%.</td>
</tr>
<tr>
<td>5-Fluorouracil (5-FU, 5%)</td>
<td>5-FU also has been FDA-approved for superficial BCCs, with similar cure rates to imiquimod.</td>
</tr>
<tr>
<td></td>
<td>Trials with more invasive BCCs are under way for both imiquimod and 5-FU. Side effects are variable, and some patients do not experience any discomfort, but redness, irritation, and inflammation are predictable.</td>
</tr>
</tbody>
</table>
The Sonic Hedgehog 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.

Wild-Type  Shh -  Shh +

![Images showing the effects of Sonic Hedgehog pathway activation and deletion in embryonic development.](image)

PNAS 2006;103:6548-6553 & Dev Biology, S. Gilbert, 10th Edition
In the absence of Hh ligand, **PTCH1 inhibits surface localization of SMO**.

Protein kinases (GSK3β) phosphorylate Gli proteins, leading to an NH2-terminal truncated form, which acts as a repressor of Hh target gene expression. **Gli3 is the predominant repressor**.

SUFU also regulates the pathway by binding to Gli, both in the cytoplasm and in the nucleus, to prevent it from activating Hh target genes.

Hh-mediated inactivation of PTCH1 allows **relocation of SMO to the tip of cilia**.

Active SMO signals downstream through an intermediary SUFU, promoting the release of Gli family transcription factors, which can then translocate to the nucleus to affect gene transcription.

Gli2 seems to be a particularly strong activator of downstream gene transcription (along with Gli1), while Gli3 is inhibitory in most contexts.

**Pathway activation and release from SUFU can lead to proteosomal degradation of Gli3 and to preferential nuclear translocation of Gli1 and Gli2**, which activate transcription of multiple target genes.
Mutated Cancer Genes and non-mutated Mediator Genes

Oncogenes and Tumor Suppressors Mutated in Cancer

- Signal Transduction: 56%
- Transcription: 35%
- Cell Adhesion: 1%

Non-mutated Mediator Genes

- Signal Transduction: 33%
- Transcription: 13%
- Cell Adhesion/motility: 13%
- Metabolism/transport: 15%
- Unknown: 15%
- Apoptosis: 8%
- Other: 3%

New targeting opportunities
IPI-926 (saridegib)  
**GDC-0449**  
(vismodegib/Erivedge)  
BMS-833923 (XL139)  
LDE-225  
NVP-LEQ506 (Smo D473H)

6% - 21%  
>60%  
4.8%
Basal cell nevus syndrome (BCNS)

- Inherit one mutated copy of the patched \((PTCH)\) gene.
- \(PTCH\) haploinsufficiency is responsible for the developmental abnormalities associated with NBCCS.
- Tumors in NBCCS individuals likely to arise with inactivation of the remaining \(PTCH\) allele.
Vismodegib, a sonic hedgehog inhibitor reduces tumor burden in BCNS

- Vismodegib reduces the tumor burden and blocks growth of new BCCs in patients with NBCCS.
ErivedgeTM (vismodegib)

- The first medicine ever for advanced BCC
- An oral drug approved by the FDA in early 2012 only for very limited circumstances where the nature of the cancer precludes other treatment options (such as surgery or radiation).
- Due to a risk of birth defects, vismodegib should not be used by women who are pregnant or attempting to conceive.
Adverse events in vismodegib-treated subjects

<table>
<thead>
<tr>
<th>Adverse Events</th>
<th>GDC-0449 (%)</th>
<th>Placebo (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Taste loss</td>
<td>83</td>
<td>8</td>
</tr>
<tr>
<td>Muscle cramps</td>
<td>70</td>
<td>8</td>
</tr>
<tr>
<td>Hair loss</td>
<td>50</td>
<td>0</td>
</tr>
<tr>
<td>Weight loss</td>
<td>50</td>
<td>8</td>
</tr>
<tr>
<td>Dropped out due to AEs</td>
<td>30</td>
<td>0</td>
</tr>
</tbody>
</table>

- **Tumor recurrence.**
- **The adverse events** associated with treatment led to discontinuation in over half of treated patients.
- **Acquired resistance:** the first documented mechanism of clinical acquired resistance to vismodegib is a secondary mutation in the extracellular domain of SMO, D473H, which prevents vismodegib binding.
# Hh pathway modulators with different mechanisms of action

| Hh pathway modulator | Mechanism of action | Comment | Status a
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></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 cyclopamine; 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 cyclopamine; 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 cyclopamine; 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 cyclopamine; 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 PFS, 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 (ALLO1)</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">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>

aHigh dose needed to achieve efficacious plasma levels. bStatus in regard to Hh-driven cancers. Bud, budesonide; Cic, ciclesonide; FA, fluocinolone acetonide; MOA, mechanism of action; PFS, prostate-specific antigen progression-free survival; TA, triamcinolone acetonide.
Acquired resistance to SMO inhibitors and approaches to overcome resistance

- Secondary mutations in SMO
- Amplification of GLI2 (or other downstream Hh pathway targets)
- Upregulation of noncanonical GLI signaling mediated by the PI3K-mTOR signaling
- Activation of aPKC
Tumor types with evidence of crosstalk between Hh and other signaling pathways

ESOPHAGEAL
PI3K/AKT/mTOR

MELANOMA
RAS/RAF/MEK/ERK
PI3K/AKT/mTOR

BCC
EGFR/RAS/RAF/MEK/ERK

BREAST
PI3K/AKT/mTOR
N OTCH

GASTRIC
RAS/RAF/MEK/ERK
PI3K/AKT/mTOR

PROSTATE
EGFR
RAS/RAF/MEK/ERK
PI3K/AKT/mTOR
N OTCH

OVARIAN
N OTCH

Glioma
EGFR
RAS/RAF/MEK/ERK
PI3K/AKT/mTOR
N OTCH

MB
EGFR
PI3K/mTOR

HNSCC
EGFR

NSCLC
EGFR

PANCREATIC
EGFR
RAS/RAF/MEK/ERK
PI3K/AKT/mTOR
N OTCH

CHOLANGIO-CARCINOMA
RAS/RAF/MEK/ERK

Can Treat Rev 40 (2014) 750-759
Melanoma

- Originates in the pigment-producing melanocytes in the basal layer of the epidermis; least common (about 1-2% of skin cancer)
- If melanoma is recognized and treated early, it is almost always curable, but if it is not, it can be **highly metastatic**, causes 75% of skin cancer deaths.
- 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).

**Warning Signs:**

**The ABCDEs of Melanoma**

- Asymmetrical
- Borders are uneven
- Multiple colors/shades
- Larger than ¼ inch

**Evolution:** changing in size, shape and color

- Melanoma is caused mainly by intense, occasional UV exposure (frequently leading to sunburn).
Melanoma can vary in appearance
The Clark Model (Hematoxylin and Eosin): Melanoma progression

<table>
<thead>
<tr>
<th>Histopathological Appearance</th>
<th>Description</th>
<th>Histologic Features</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benign nevus</td>
<td><strong>Step 1</strong></td>
<td>Proliferation of melanocytes leading to the benign nevus. Clinically, these nevi present as flat or slightly raised lesions with either uniform coloration or a regular pattern of dot-like pigment in a tan or dark brown background. Histologically, such lesions have an increased number of nested melanocytes along the basal layer (arrows).</td>
</tr>
<tr>
<td>Dysplastic nevus</td>
<td><strong>Step 2</strong></td>
<td>Dysplastic cells Random atypia</td>
</tr>
<tr>
<td>Radial-growth phase</td>
<td><strong>Step 3</strong></td>
<td>Intraepidermal growth Continuous atypia</td>
</tr>
<tr>
<td>Vertical-growth phase</td>
<td><strong>Step 4</strong></td>
<td>Dermal invasion</td>
</tr>
<tr>
<td>Metastatic melanoma</td>
<td><strong>Step 5</strong></td>
<td>Metastasis</td>
</tr>
</tbody>
</table>

Lesions that progress to the vertical-growth phase acquire the ability to invade the dermis and form an expansile nodule, widening the papillary dermis. The cells can also extend into the reticular dermis and fat, are capable of growth in soft agar, and have the capacity to form tumor nodules when implanted in nude mice.

The final step in the model is the successful spread of cells to other areas of the skin and other organs, where they can successfully proliferate and establish a metastatic focus. These cells can grow in soft agar and can form tumor nodules that may metastasize when implanted in nude mice.
Molecular Changes in the Progression of Melanoma

<table>
<thead>
<tr>
<th>Stage</th>
<th>Benign Nevus</th>
<th>Dysplastic Nevus</th>
<th>Radial-Growth Phase</th>
<th>Vertical-Growth Phase</th>
<th>Metastatic Melanoma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epidermis</td>
<td></td>
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<tr>
<td>Basement membrane</td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dermis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Biologic Events</th>
<th>Benign</th>
<th>Premalignant</th>
<th>Decreased differentiation</th>
<th>Vertical-Growth Phase</th>
<th>Metastatic Melanoma</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Limited growth</td>
<td>Lesions may regress</td>
<td>Unlimited hyperplasia Cannot grow in soft agar Clonal proliferation</td>
<td>Crosses basement membrane Grows in soft agar Forms tumor</td>
<td>Dissociates from primary tumor Grows at distant sites</td>
</tr>
</tbody>
</table>

| Molecular Lesions    | B\(\text{RAF}\) mutation | CDKN2A loss - PTEN loss | Increased CD1 | E-cadherin loss | N-cadherin expression | \(\alpha\)\(\text{v}\)\(\beta\)3 integrin expression | MMP-2 expression | Survivin | Reduced TRPM1 | Absent TRPM1 |
|----------------------|---------------------------|------------------------|--------------|-----------------|----------------------|-----------------|----------|-------------|-------------|

CD1: cell surface glycoproteins and represent an antigen-presenting system: decreased in metastatic melanomas.

Decreased expression of melanoma markers regulated by microphthalmia-associated transcription factor (MITF).

Striking changes in the control of cell adhesion.

Changes in the expression of the melanocyte-specific gene melanostatin 1 (TRPM1) correlate with metastatic propensity, but the function of this gene remains unknown.
Determinants of melanoma tumorigenesis

Two large cancer circuits in melanoma—the tumor-constraining **CDKN2A network** and the growth-promoting **Ras signaling network**—and their interaction with a key downstream regulator of apoptosis, the Bcl-2/p53 network.
Familial melanomas are associated with highly penetrant germline mutations in cyclin-dependent kinase (CDK) inhibitor 2A (CDKN2A), a gene that codes for two different tumor suppressors: p16INK4a and p14ARF (Goldstein et al., 2007).

Other highly penetrant mutations occur in CDK4 and BRCA1-associated protein 1, BAP1.

Germline CDKN2A mutations were identified in 25-50% of familial melanoma kindreds and in up to 10% of individuals with multiple primary melanomas.

Somatic alterations in up to 30-70% of sporadic melanomas.

The majority of melanoma cases would be sporadic rather than familial.
NRAS

- Activating NRAS mutations are relatively common (26%) in sporadic melanoma.
- Oncogenic NRAS is capable of inducing melanoma in CDKN2A-deficient mice.
- Oncogenic HRAS has been shown to play a key role in melanoma maintenance in murine models. Although NRAS is now accepted to be a key oncogene in human melanoma, other members of the RAS family of proteins have a limited role in humans. Among melanocytic tumors, HRAS alterations have been found in benign Spitz nevi.
- As Ras activates both the MAPK and PI3K pathways, it is thought that redundant downstream activating mutations do not provide additional survival benefits and may in fact be cytotoxic.
- Indeed, mutations in NRAS have been demonstrated to occur in a reciprocal pattern with genes downstream of it in either pathway, such as PTEN and BRAF.
- NRAS mutations are more frequent on chronically sun-exposed sites. However, the most common Gln61 alterations in NRAS are not classic UVB-signature changes and NRAS mutations appear to be at least as common in congenital nevi as melanoma, indicating that sun exposure is not necessary to induce the most common NRAS mutations.
- These data suggest that oncogenic NRAS, which arises by a yet-to-be-determined molecular mechanism(s), promotes melanocytic proliferation but is not sufficient to yield true malignant transformation.
Ras signaling network

- Regulates cell growth, survival, and invasion through two distinct cascades—the Ras/mitogen-activated protein kinase (MAPK) and the Ras/phosphatidylinositol-3-kinase (PI3K) signaling streams.
BRAF belongs to the RAF family of serine/threonine protein kinases: ARAF, CRAF, and BRAF, of which the latter has the highest basal kinase.

Active RAS acts via adaptor proteins to activate and recruit RAF to the cell membrane where they are activated.

Active BRAF signals through MEK to activate ERK and downstream signaling to induce a range of biochemical processes including cell differentiation, proliferation, growth, and apoptosis.

**BRAF is the most potent activator of MEK.** ARAF and CRAF require an additional phosphorylation in the N-region of their kinase domain for full activation, likely contributing to the predominance of the BRAF isoform in the activation of MEK.

EGFR overexpression:
- Colorectal cancer (27-77%)
- Pancreatic cancer (30-50%)
- Lung cancer (40-80%)
- Non-small cell lung cancer (14-91%)

BRAF V600E mutation
- Constitutively active kinase, oncogenic addiction
- Overactivation of ER pathway
- cell proliferation
BRAF is a key element in melanoma tumorigenesis and crucial therapeutic target

- BRAF is mutated in a wide range of cancers (e.g., colorectal, thyroid, ovarian, breast, and lung cancers), but it is altered in 80% of short-term melanoma cell cultures and 66% of uncultured melanomas thereby making BRAF the single most commonly mutated gene in melanoma.

- About 90% of the reported changes occur at a single codon in the BRAF kinase domain (Threonine1799Alanine, Valine600Glutamic acid). BRAF\textsuperscript{V600E} can gain 500-fold increased activation.

- BRAF\textsuperscript{V600E} is prevalent in benign nevi supporting a role for BRAF activation in melanocytic proliferation, but not full transformation.

- BRAF activation leads only to development of benign nevi, whereas progression to frank melanoma requires concurrent p53 inactivation. Furthermore, oncogenic BRAF has been shown to result in a senescence-like state (via p16INK4a/p14ARF). The full oncogenic potential of BRAF appears to be dictated by the presence or absence of other genetic constraints.

- Patients that possess germline mutations in p16INK4a/p14ARF have increased susceptibility to melanoma.
Unclear carcinogenic mechanism underlying the BRAFV600E mutation

The T>A transversion is not a classic UVB signature change and many internal malignancies harbor the identical BRAF alteration, thus UV radiation is clearly not essential for c.T1799 mutagenesis.

Moreover, the c.T1799A mutation has been shown to occur most frequently on tumors arising from intermittently sun-exposed sites and not on chronically sun-damaged skin, where UVB exposure is the most intense.

It has been suggested that UVB-induced cyclobutane pyrimidine dimer formation at the neighboring position (c.1800–1801) may promote subsequent BRAF mutations at the 1799 position.
ERK signaling under physiologic conditions and in tumors harboring BRAF V600E mutations

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, involving conformational changes, phosphorylation and dimerization.
- MEK activation
- ERK activation

- Regulation of a number of cytoplasmic and nuclear substrates, including several TFs that control genes responsible for cell cycle progression and proliferation.

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

- ERK regulates the expression of genes encoding proteins such as SPRY and DUSPs; both of which have an indirect negative-feedback effect on pathway activity (dotted lines).

- SPRY proteins are thought to impede signaling by disrupting the GRB2-SOS interaction, and DUSPs are ERK-specific phosphatases.
In tumors harboring a BRAF V600E mutation, hyperactivated ERK signaling results in increased proliferation and evasion of apoptosis.

ERK-dependent negative feedback suppresses RTK-mediated signaling, resulting in low amounts of active GTP-bound RAS.

In this state, BRAF V600E signals as a functional monomer.
Clinical efficacy of PLX4032 (Vemurafenib), a BRAF inhibitor

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)
Clinical effects of PLX4032 (Vemurafenib)

- Strong initial effects
- Drug resistance
- Tumor recurrence
Mechanisms of resistance to RAF inhibition

a  RAF dimerization–dependent mechanisms

Alterations that promote enhanced RAF dimerization such as

- NRAS mutation (NRAS Q61),
- expression of RAF splice variants (p61), and
- CRAF overexpression cause resistance to RAF inhibitors.

b  RAF dimerization–independent mechanisms

Reactivation of ERK signaling and resistance to RAF inhibitors may also occur in a dimerization-independent fashion as a result of downstream mutations in MEK or RAF bypass resulting from activation of COT (an ERK kinase kinase).
Adaptation to RAF inhibitors

Hyperactivated ERK signaling
Negative feedback suppression of RAS-GTP
BRAF signals as a monomer

RAF inhibitor
Inhibition of ERK output and signaling

Inhibition of ERK feedback
Restoration of RTK signaling/increase of RAS-GTP
Formation of inhibitor-insensitive RAF dimers
Reactivation of ERK signaling

Partial restoration of negative-feedback pathways
New steady state of reactivated ERK signaling

Nat Med 19 (2013) 1401
TREATMENT

Chemotherapy:

• Dacarbazine (DTIC; a member of the class of alkylating agents) given by injection. DTIC may be combined with carmustine (BCNU; a mustard gas-related) and tamoxifen, or with cisplatin and vinblastine. Temozolomide, an oral drug closely resembling DTIC, is FDA-approved for brain cancers but also used off-label for melanomas that have spread to the brain or nervous system.

• Studies are under way with the anti-angiogenic drug thalidomide, combined with the chemotherapeutic agent, temozolomide. Angiostatin and endostatin have shown some degree of efficacy against melanoma in preliminary studies.

Immunotherapy/biochemotherapy:

• Injectable interferon (IFN) alpha-2b, the only drug with FDA approval to treat “high-risk” melanomas. IFN alpha-2b appeared to increase disease-free interval to an average of 9 months, but did not lengthen overall survival.

• Tumor necrosis factor (tumor-killing) factor

• ipilimumab (MDX-010 or MDX-101), a monoclonal antibody that has been tested in clinical trials for advanced Stage III and IV melanoma. immune-stimulating treatment

• imatinib (Gleevec), which inhibits c-KIT, the receptor for tyrosine kinase. significant clinical improvements from the drug as a single therapy have been minimal.

• PLX-4032 (a BRAF inhibitor) received the most notable success in targeted melanoma therapy.
Disruption of Multiple signaling networks

I. The growth-promoting Ras signaling network

II. The tumor-constraining CDKN2A network

III. PI3K-AKT cascade: promoter of melanoma progression

The expression of PI3K and AKT have been shown to steadily increase during the progression from benign nevi to early melanoma, and to metastatic disease.

Malignant melanoma is frequently driven by mutational activation of BRAF accompanied by silencing of the PTEN tumor suppressor.
Key issues in skin cancer research and therapy

- Tumor Recurrence
- Drug-induced skin cancers

Immunosuppression
(calcineurin inhibitors)
transplant recipients

- CsA increase Akt activation.
- Akt hyperactivation is essential for both growth and survival of CsA-treated cells.

TNF Inhibition
(remicade/Enbrel/Humira)
rheumatoid arthritis
psoriasis

- ↓ Inflammation

BRAF Inhibition
melanoma

Assessing Skin Cancer Risk in Patients with RA and Psoriatic Arthritis
Richard G. B. Langley, MD, FRCP, Professor of Medicine (Dermatology), Division of Dermatology, Department of Medicine, Dalhousie University; Kathryn Dao, MD, Baylor Research Institute; John J. Cush, MD, Baylor Research Institute.

Rheumatoid arthritis and psoriasis patients are at increased risk for non-melanoma skin cancers (NMSC), and TNF antagonists may increase the risk further. This association has resulted in use was associated with a higher rate of melanoma recurrence (3/17 or 18%), compared to no recurrence in those treated with DMARDs (0/10).80

- ↓ Inflammation

RAF inhibition and induction of cutaneous squamous cell carcinoma
Caroline Robert, Jean-Philippe Arnault and Christine Mateus

- Ras-dependent BRAF/CRAF activation
RAF/MEK/ERK Inhibition
Advanced renal cell carcinoma
Advanced hepatocellular carcinoma
Thyroid cancer

Sorafenib: A drug approved for the treatment of primary kidney cancer (advanced renal cell carcinoma), advanced hepatocellular carcinoma, and Thyroid Cancer.

↑ skin cancer during sorafenib treatment for advanced RCC; the median time from the start of sorafenib therapy until observation of a skin cancer lesion was 13.5 months.