The Role of HHLA2 in Non-Small Cell Lung Cancer

Name of student
Off-Topic Candidacy Exam
Date of Exam
Advisor: Name of Advisor
Lung Cancer Statistics

- Lung cancer is the second most common cancer in the USA, with ~200,000 new cases annually.

<table>
<thead>
<tr>
<th>Estimated Deaths</th>
<th>Males</th>
<th>Females</th>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>84,590</td>
<td>27%</td>
<td>71,280</td>
<td>25%</td>
</tr>
<tr>
<td>Lung &amp; bronchus</td>
<td>Colon &amp; rectum</td>
<td>Prostate</td>
<td>Pancreas</td>
<td>Breast</td>
</tr>
<tr>
<td></td>
<td>27,150</td>
<td>26,730</td>
<td>22,300</td>
<td>40,610</td>
</tr>
<tr>
<td></td>
<td>9%</td>
<td>8%</td>
<td>7%</td>
<td>14%</td>
</tr>
<tr>
<td>Colon &amp; rectum</td>
<td>Prostate</td>
<td>Pancreas</td>
<td>Breast</td>
<td>Colon &amp; rectum</td>
</tr>
<tr>
<td></td>
<td>27,150</td>
<td>22,300</td>
<td>40,610</td>
<td>23,110</td>
</tr>
<tr>
<td></td>
<td>9%</td>
<td>7%</td>
<td>14%</td>
<td>8%</td>
</tr>
<tr>
<td>Prostate</td>
<td>Pancreas</td>
<td>Breast</td>
<td>Colon &amp; rectum</td>
<td>Pancreas</td>
</tr>
<tr>
<td></td>
<td>26,730</td>
<td>22,300</td>
<td>40,610</td>
<td>20,790</td>
</tr>
<tr>
<td></td>
<td>8%</td>
<td>7%</td>
<td>14%</td>
<td>7%</td>
</tr>
<tr>
<td>Pancreas</td>
<td>Breast</td>
<td>Pancreas</td>
<td>Colon &amp; rectum</td>
<td>Pancreas</td>
</tr>
<tr>
<td></td>
<td>22,300</td>
<td>22,300</td>
<td>40,610</td>
<td>20,790</td>
</tr>
<tr>
<td></td>
<td>7%</td>
<td>7%</td>
<td>14%</td>
<td>7%</td>
</tr>
<tr>
<td>Liver &amp; intrahepatic bile duct</td>
<td>Liver &amp; intrahepatic bile duct</td>
<td>Liver &amp; intrahepatic bile duct</td>
<td>Liver &amp; intrahepatic bile duct</td>
<td></td>
</tr>
<tr>
<td></td>
<td>19,610</td>
<td>19,610</td>
<td>19,610</td>
<td>19,610</td>
</tr>
<tr>
<td></td>
<td>6%</td>
<td>6%</td>
<td>6%</td>
<td>6%</td>
</tr>
<tr>
<td>Leukemia</td>
<td>Ovary</td>
<td>Uterine corpus</td>
<td>Leukemia</td>
<td></td>
</tr>
<tr>
<td></td>
<td>14,300</td>
<td>14,080</td>
<td>10,200</td>
<td>10,920</td>
</tr>
<tr>
<td></td>
<td>4%</td>
<td>5%</td>
<td>4%</td>
<td>4%</td>
</tr>
<tr>
<td>Esophagus</td>
<td>Uterine corpus</td>
<td>Leukemia</td>
<td>Liver &amp; intrahepatic bile duct</td>
<td></td>
</tr>
<tr>
<td></td>
<td>12,720</td>
<td>10,920</td>
<td>9,310</td>
<td>8,690</td>
</tr>
<tr>
<td></td>
<td>4%</td>
<td>4%</td>
<td>3%</td>
<td>3%</td>
</tr>
<tr>
<td>Urinary bladder</td>
<td>Liver &amp; intrahepatic bile duct</td>
<td>Non-Hodgkin lymphoma</td>
<td>Non-Hodgkin lymphoma</td>
<td></td>
</tr>
<tr>
<td></td>
<td>12,240</td>
<td>9,310</td>
<td>8,690</td>
<td>7,080</td>
</tr>
<tr>
<td></td>
<td>4%</td>
<td>3%</td>
<td>3%</td>
<td>3%</td>
</tr>
<tr>
<td>Non-Hodgkin lymphoma</td>
<td>Brain &amp; other nervous system</td>
<td>Brain &amp; other nervous system</td>
<td>Brain &amp; other nervous system</td>
<td></td>
</tr>
<tr>
<td></td>
<td>11,450</td>
<td>7,080</td>
<td>7,080</td>
<td>7,080</td>
</tr>
<tr>
<td></td>
<td>4%</td>
<td>3%</td>
<td>3%</td>
<td>3%</td>
</tr>
<tr>
<td>Brain &amp; other nervous system</td>
<td>All Sites</td>
<td>All Sites</td>
<td>All Sites</td>
<td></td>
</tr>
<tr>
<td></td>
<td>9,620</td>
<td>282,500</td>
<td>282,500</td>
<td>100%</td>
</tr>
<tr>
<td></td>
<td>3%</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
</tr>
</tbody>
</table>

Siegel et al., CA: A Cancer Journal for Clinicians, 2017
The Biology of Lung Cancer

• Understanding histology and molecular landscape of NSCLC provides basis for treatment decisions.
Rationale for Targeting Specific Driving Mutations

EGFR TKIs Show More Benefit in EGFR Mutant Patients

EGFR mut: 67%; EGFR WT: 9%; P < .0001

Mut = mutant; WT = wild type
Issues of Specific TKIs

• Only patients who harbor the specific mutations can benefit from TKIs. (He et al., Med Sci Monit. 2016)

• Resistance to TKIs often occur, leading to relapse. (Reviewed in Neel, Nature, 2017)
  • 70% of patients who harbor EGFR mutations will have a prolonged PFS of about one year, then gain resistance and relapse.
Immunotherapy Can Reverse Tumor Immune Escape

Sharma et al., NRC, 2011
Blocking CTLA-4 is Therapeutically Efficacious in Melanoma

- The CTLA-4 blocking antibody was FDA approved for treatment of metastatic melanoma in 2011.

Hodi et al., NEJM, 2010

(27.8 months vs 17.2 months, hazard ratio 0.68, p<0.001).
Blocking PD-1 is Therapeutically Efficacious in Lung Cancer

- PD-1 blocking antibody, Nivolumab was FDA approved for treatment of advanced lung cancer in 2015.

Brahmer et al., NEJM, 2015
Patients Treated with Both Specific TKIs and Immunotherapy Do Relapse

- There is an unmet need of alternative therapies for patients who relapse from initially beneficial drugs.
  - New Targets
  - Combination Therapies
**HHLA2 is a Recently Discovered T-cell Co-inhibitory Molecule**

T-cells from PBMCs cultured with an HHLA2 peptide led to lower proliferation of both CD4 and CD8 T-cells compared to the control peptide.

Janakarim et al., 2015, CCR

Zhao et al., 2013, PNAS
HHLA2 Is Expressed in Many Different Cancers

Janakiram et al., 2015, CCR
HHLA2 is Associated with Poor Survival and Metastasis in Osteosarcoma

A high expression level compared to a low expression level of HHLA2 is associated with a lower rate of survival in osteosarcoma.

A higher expression rate of HHLA2 is also associated with metastasis in osteosarcoma.

Koirala, 2016, Sci Rep
HHLA2 is Widely Expressed in Lung Cancer

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Discovery cohort (n = 392)</th>
<th>Validation cohort (n = 287)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HHLA2 Negative</td>
<td>HHLA2 Positive</td>
</tr>
<tr>
<td>Age, year</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female (n = 215)</td>
<td>78 (36%)</td>
<td>137 (64%)</td>
</tr>
<tr>
<td>Male (n = 141)</td>
<td>55 (39%)</td>
<td>86 (61%)</td>
</tr>
<tr>
<td>Histology</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adeno (n = 290)</td>
<td>91 (31%)</td>
<td>199 (69%)</td>
</tr>
<tr>
<td>Squam (n = 31)</td>
<td>20 (65%)</td>
<td>11 (35%)</td>
</tr>
<tr>
<td>Large (n = 18)</td>
<td>16 (89%)</td>
<td>2 (11%)</td>
</tr>
<tr>
<td>Stage</td>
<td></td>
<td></td>
</tr>
<tr>
<td>I (n = 252)</td>
<td>85 (34%)</td>
<td>167 (66%)</td>
</tr>
<tr>
<td>II (n = 47)</td>
<td>23 (49%)</td>
<td>24 (51%)</td>
</tr>
<tr>
<td>III (n = 35)</td>
<td>10 (29%)</td>
<td>25 (71%)</td>
</tr>
<tr>
<td>Mutation status</td>
<td></td>
<td></td>
</tr>
<tr>
<td>EGFR (n = 41)</td>
<td>10 (24%)</td>
<td>31 (76%)</td>
</tr>
<tr>
<td>KRAS (n = 62)</td>
<td>23 (37%)</td>
<td>39 (63%)</td>
</tr>
<tr>
<td>WT/WT (n = 91)</td>
<td>43 (47%)</td>
<td>48 (53%)</td>
</tr>
</tbody>
</table>

Cheng et al, 2017, CCR
Central Hypothesis

Targeting HHLA2 in NSCLCs will be effective for killing cancer cells through a cytotoxic T-cell mediated mechanism of action.
Specific Aims

**Aim 1**: Analyze the role of HHLA2 on T-cells within the tumor microenvironment.

**Aim 2**: Determine the therapeutic efficacy of targeting HHLA2 in NSCLC.
Specific Aim 1

Analyze the role of HHLA2 on T-cells within the tumor microenvironment.

1. Analyze the T-cells of the TME in NSCLC based on HHLA2 expression.
2. Examine the changes of T-cell sub-populations when HHLA2 is blocked within the TME.
3. Investigate the activation of tumor infiltrating lymphocytes (TILs) or splenocytes of HHLA2 expressing NSCLC bearing mice.
Specific Aim 2

Determine the therapeutic efficacy of targeting HHLA2 in NSCLC.

1. Test the therapeutic effect of a blocking HHLA2 in NSCLC.
2. Investigate the therapeutic effect of targeting HHLA2 and PD-L1 \textit{in vivo}.
3. Inspect therapeutic effectiveness of blocking EGFR and HHLA2 in \textit{EGFR} mutant NSCLC \textit{in vivo}.
Human Lung Cancer Models

• NSCLC tumor biopsies will be collected from the MD Anderson Department of Thoracic Head and Neck Medical Oncology.

• The Biopsy will be split into three portions
  1. IHC of HHLA2
  2. Flow cytometry of fresh biopsy to analyze T-cell subpopulations of the TME
  3. Development of lung cancer cell lines and matched T-cells
Aim 1.1: Analyze the T-cells of the TME in NSCLC based on HHLA2 expression.

- **Materials:** Lung Cancer Biopsies, Human NSCLC Cells and Matched T-Cells

- **Methods:**
  - **HHLA2 Expression:** IHC and Flow Cytometry
  - **Analysis of T-cell Subpopulations:** Flow Cytometry

**Common T-Cell Markers**
- CD45
- CD3
- CD4 or CD8

**Exhausted T-Cells**
- PD-1
- TIM3
- LAG-3

**Proliferative and Active T-Cells**
- Ki67
- Granzyme B
- CD38

**Regulatory T-Cells**
- CD25
- FoxP3
Aim 1.1: Expected Results

HHLA2

Score 1 | Score 2 | Score 3 | Score 4
---|---|---|---
No Expression | Low | Intermediate | High
0% | 1-15% | 16-30% | 30%+

Acquired from Chiou et al., Scientific Reports, 2017

HHLA2 Expression: Stage III and IV NSCLC

- 32% Negative (16/50)
- 68% Positive (34/50)

HHLA2 IHC Score: Stage III and IV NSCLC

- 32% Score 1 (16/50)
- 6% Score 2 (3/50)
- 25% Score 3 (13/50)
- 36% Score 4 (18/50)
Aim 1.1: Expected Results

Percentage of Proliferative/Active T-Cells in Non- vs. High HHLA2 Expressing Tumors

Percentage of PD1, TIM3, or LAG3 Exhausted T-Cells in Non- vs. High HHLA2 Expressing Tumors

Regulatory T-Cells in Non- vs. High HHLA2 Expressing Tumors
Aim 1.2: Examine the changes of T-cell sub-populations when HHLA2 is blocked within the TME.

- **Model:** Human NSCLC Cells and Matched T-Cells
- **Method:** Genetically Modify NSCLC cell lines

**Analysis of T-Cell Subpopulations:**
Flow Cytometry: CFSE and Markers of Ki67, Granzyme B, CD38, PD-1, TIM3, LAG-3, CTLA-4, CD25, and FoxP3
Aim 1.2: Expected Results

**Percent of Non-Proliferating T-Cells**

- **Control** vs **HHLA2-KO** (High HHLA2 Expression)
- **Control** vs **HHLA2-OE** (Non HHLA2 Expression)

**Percent of CD4+ or CD8+ Proliferative (CD38+/Ki67+/Granzyme B+) T-Cells**

- **CD4**
- **CD8**

**Percent of CD4+/CD25+/FOXP3+ Regulatory T-Cells**

- **Control** vs **HHLA2-KO** (High HHLA2 Expression)
- **Control** vs **HHLA2-OE** (Non HHLA2 Expression)

**Percent of CD4+ or CD8+ Exhausted T-Cells**

- **High HHLA2 Expression**
- **Non HHLA2 Expression**

[Graphs showing statistical significance with **** for p < 0.0001]
Aim 1.3: Investigate the activation of tumor infiltrating lymphocytes (TILs) or splenocytes of NSCLC bearing mice.
**In Vivo Model**

- HHLA2 is expressed in humans, but not in mice.
- Humanized NSG mice

Modified from the Jackson Laboratory
Aim 1.3: Investigate the activation of tumor infiltrating lymphocytes (TILs) or splenocytes of NSCLC bearing mice.

- **Model:** Humanized NSCLC Mouse Model
- **Method:** Co-Culture Experiments

**ELISA**
1. IFN$\gamma$
2. IL-2
Aim 1.3: Expected Results

- The highest levels of IFNγ and IL-2 will be in the cells co-cultured with TILs or splenocytes from the mice implanted with the non-HHLA2 expressing cells.
Aim 1: Potential Pitfalls and Alternatives

- T-cell inactivation might be employed by PD-L1/PD-1 or CD80/CD86/CTLA-4 signaling, and cause HHLA2 inactivation of T-cells to be unrecognized.
  - PD-L1, PD-1, and CTLA-4 expression and function will be sought out as necessary.
  - Genetic or therapeutic blockage of PD-L1, PD-1, or CTLA-4 will be used.

- HHLA2 might be a silenced immune evasion mechanism.
  - Resistance models will be sought out.
Aim 2.1: Test the therapeutic effect of a blocking HHLA2 in NSCLC.

- **Model:** Human NSCLC Cells and Matched T-Cells

- **Methods:**
  - **Measure Cell Growth:** CyQUANT Assay
  - **Apoptosis:** Annexin Assay

**HHLA2 Tumor Expression**

- **High-**
  - Anti-CD3 + Control Ig
  - Anti-CD3 + anti-HHLA2 Ig

- **Intermediate-**
  - Anti-CD3 + Control Ig
  - Anti-CD3 + anti-HHLA2 Ig

- **Non-**
  - Anti-CD3 + Control Ig
  - Anti-CD3 + anti-HHLA2 Ig
**In Vivo Model**

- HHLA2 is expressed in humans, but not in mice.
- Humanized NSG mice

---

**Developed NSCLC Cell Lines**

<table>
<thead>
<tr>
<th>HHLA2 Expression</th>
<th>Implantation Locations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non</td>
<td>1. Orthotopic</td>
</tr>
<tr>
<td>Intermediate</td>
<td>2. Subcutaneous (Flank)</td>
</tr>
<tr>
<td>High</td>
<td></td>
</tr>
</tbody>
</table>

**Implantation Locations**

- 1. Orthotopic
- 2. Subcutaneous (Flank)

**Control Antibody**

**HHLA2 Blocking Antibody**

---

Modified from the Jackson Laboratory
Aim 2.1: Expected Results

- I hypothesize that the HHLA high expressing cancer cells cultured with the HHLA2 blocking antibody will undergo less proliferation and more cell death compared to the control Ig group.
Aim 2.1: Expected Results

- I hypothesize that the HHLA2 blocking antibody will extend survival and yield long-term survivors.
Aim 2.1: Expected Results

- I hypothesize that the HHLA2 blocking antibody will slow down tumor growth.
Aim 2.2: Investigate the therapeutic effect of targeting HHLA2 and PD-L1 in vivo.

- Model: Humanized NSG Mouse Model
- Methods: Survival Experiments and Tumor Measurements

- Developed NSCLC Cell Lines (PD-L1 +)
  - HHLA2 Expression:
    - Non
    - Intermediate
    - High
- Control Antibody
- HHLA2 Blocking Antibody
- PD-L1 Blocking Antibody
- Combo

Implantation Locations:
- 1. Orthotopic
- 2. Subcutaneous (Flank)
Aim 2.2: Expected Results

- I hypothesize that targeting both HHLA2 and PD-L1 in an HHLA2-overexpressing NSCLC model will extend the survival and yield long-term survivors compared to other treated groups.
Aim 2.2: Expected Results

- I hypothesize that targeting both HHLA2 and PD-L1 in an HHLA2-overexpressing NSCLC model will delay growth of the tumor compared to other treated groups.
Aim 2.3: Inspect therapeutic effect of blocking EGFR and HHLA2 in EGFR mutant NSCLC in vivo.

- **Model:** Humanized Lung Cancer Mouse Model
- **Methods:** Survival Experiments and Tumor Measurements

![Diagram showing experimental setups](image-url)

- Developed NSCLC Cell Lines (HHLA2+ and PD-L1+)
  - EGFR Status: WT, Mutant
  - Control Antibody
  - HHLA2 Blocking Antibody
  - EGFR TKI
  - Combo

- Implantation Locations
  - 1. Orthotopic
  - 2. Subcutaneous (Flank)
Aim 2.3: Expected Results

- I hypothesize that treatment using an EGFR TKI and an HHLA2 blocking antibody in a EGFR mutant NSCLC model will extend the survival of the mice compared to the other treatment groups based on the theory that HHLA2 is highly expressed on EGFR mutant cells for tumor evasion.

**Survival of NSCLC Bearing Hu-NSG Mice**

- **EGFR WT**
  - Control
  - Anti-HHLA2
  - EGFR TKI
  - Combo

- **EGFR Mutant**
  - Control
  - Anti-HHLA2
  - EGFR TKI
  - Combo
Aim 2.3: Expected Results

**Mean Tumor Volume of WT-EGFR/HHLA2+ NSCLC Bearing Hu-NSG Mice**

**Mean Tumor Volume of Mutant-EGFR/HHLA2+ NSCLC Bearing Hu-NSG Mice**
Aim 2.3 Extension: Inspect therapeutic effect of blocking EGFR, PD-L1, and HHLA2 in EGFR mutant NSCLC \textit{in vivo}.

- **Model:** Orthotopic Lung Cancer Mouse Model
- **Methods:** Survival Experiments and Tumor Measurements
Aim 2.3 Extension: Expected Results

Survival of EGFR Mutant/PD-L1+/HHLA2+ NSCLC Bearing Hu-NSG Mice

![Graph showing survival rates for different treatments:]

- **Control**
- **Anti-HHLA2**
- **EGFR TKI**
- **Anti-PD-L1**
- **Anti-HHLA2/Anti-EGFR**
- **Anti-HHLA2/Anti-EGFR/Anti-PD-L1**

**Percent Survival**

**Days Elapsed**

EGFR Mutant/PD-L1+/HHLA2+
Aim 2: Potential Pitfalls and Alternatives

- Genetic variability might be seen within the PDX models.
  - Use of the genetically modified models of Aim 1.2 will be utilized instead.

- HHLA2 activation might arise in response to anti-PD-L1 or anti-EGFR treatment.
  - Anti-PD-L1 or anti-EGFR resistant models will be developed.
  - Sequential treatment of anti-PD-L1 or anti-EGFR followed by anti-HHLA2 will be utilized.
Alternative Strategies

• Explore other aspects of the TME.
  • MDSCs, B-Cells, NK Cells

• Block other members of the B7 family, B7x or B7-H3, in NSCLC.
Conclusions

• HHLA2 is a recently discovered member of the B7 family of T-cell inhibitory molecules.

• HHLA2 is widely expressed in NSCLC, and may provide a novel therapeutic immuno-target.

• Successful results from targeting HHLA2 in NSCLC immunocompetent mouse models may lead to clinical trials and exploration of HHLA2 in other cancers.
Thank You!
### Development of lung cancer cell lines and matched T-cells

1. **Lung Cancer Patient**
   - Biopsy
   - Culture Cancer Cells in complete RPMI
   - Expand + TILs
   - TIL Enrichment: Culture with IL-2

#### Common T-Cell Markers:
- CD45, CD3, CD8 or CD4

#### Proliferative and Active T-Cells
- Ki67
- Granzyme B
- CD38

#### Exhausted T-Cells
- PD-1
- TIM3
- LAG3

#### Regulatory T-Cells
- CD25
- FoxP3

Characterize TILs by FACS Analysis
The Human Lung Cancer Model: Cancer Cells with Matched T-Cells
Rapid Expansion

GMP MANUFACTURING FACILITY

Initial TIL Culture (Pre-REP) 3 weeks

+IL-2

Bulk TILs (>75M)

Rapid Expansion Protocol (REP) 2 weeks

+IL-2 + OKT3 + feeder cells

Infusion bag

Final TILs

≥1.5 cm diameter

Excise Tumor

Overnight Shipping

IV infusion + IL-2

Overnight Shipping
Smoker
Symptoms like cough, pain, blood in sputum,

Glandular Bronchioles
Non-Smoker

central part of lung in one of main airways
Smoker
Symptoms like cough, pain, blood in sputum,
**Lung Cancer Treatment Algorithm**

**Figure 2** | Treatment algorithms for non-small cell lung cancer patients whose tumors do not have *EGFR* or *ALK* mutations (wild-type). (A) Current treatment algorithm. (B) Future treatment algorithm.

Melosky, Frontiers in Oncology, 2017
HHLA2 Signaling

A. B7 Family
   - HHLA2
   - B7-1
   - B7-2
   - ICOS-L
   - PD-L1
   - PD-L2
   - B7x
   - B7-H3

CD28 Family
   - CD28
   - CTLA-4
   - ICOS
   - PD-1
   - TMIGD2

B. Angiogenesis
   - Endothelial Cells
   - HHLA2
   - TAM
   - Tumor Cell
   - Coinhibition

Other Immune Cells

= IgC like domain
= IgV like domain
= Unidentified
= Unidentified

Janakiram, Oncoimmunology, 2015
A Putative Receptor Exists for HHLA2
Loss of Tumor Suppressor Genes (TSG) in Progression in Colon Carcinoma

(See Also Sidebar 11.1, p. 434
Relating p53 loss to RAS mutations in
the same cancer cell.)

“DCC” Gene = Deleted in Colon Carcinoma.
Identity not known.

“APC” = Adenomatous polyposis coli gene (Cancer suppressor gene)
“K-ras” = Oncogene activated, transduced, or mutated, first identified in virally-induced rat sarcoma. (On chromosome 1*)
TSG = Tumor Suppressor Gene
p53 = Major cancer suppressor gene

PMCID: PMC555446
Localisation of the human N-ras oncogene to chromosome 1cen - p21 by in situ hybridisation.
M Davis, S Malcolm, A Hall, and C J Marshall
Clonal Expansion of Cancer
Hallmarks of Cancer
The cell cycle and implications for cancer genetics

The cyclins and CDKs promote cell cycle progression, while the CDK inhibitors stop it. The balance between the two groups of molecules determines whether the cell proliferates or is quiescent.

CDK inhibitors stop progression of the cell cycle by inhibiting the cyclins and CDKs. They act at multiple phases of the cell cycle and are especially important at the G1/S and G2/M checkpoints.

CDK inhibitors inhibit cell cycle progression by inactivating the cyclins and CDKs. This can lead to cell cycle arrest or cell death.

The early phase of G1 (the first gap phase) is mitogen-dependent. The presence of growth factors is required for progression beyond this point. Once the cell progresses past the R point, mitogens are no longer required for cycle progression.

Quiescence: cells can exit and re-enter the cell cycle from G0 phase.

The late phase of G2 is characterized by DNA replication and preparation for mitosis.

DNA replication occurs in the S (synthesis) phase. The G1/S checkpoint allows checking of DNA integrity before cell division.

Oncogenes
The lack of inhibition or gain of function of which will lead to cancer.
- Cyclins
- Cyclin-dependent kinases (CDKs)
- E2F
- MYC
- Mdm2

Tumour suppressors
The increased inhibition or loss of function of which will lead to cancer.
- RB
- p53
- p21, p27, p57 (CIP/KIP/WAF)
- p14/ARF
- p16/INK4a, p17, p18, p19
- TGF β receptor
- BRCAl, BRCAl2
- ATM
Cancer Cell Signaling
T-Cell Signal Transduction
- **Neurocrine**—secretion of hormones into the bloodstream by neurons
- **Endocrine**—secretion of hormones into the bloodstream by endocrine glands
- **Paracrine**—hormone molecule secreted by one cell affects adjacent cells
- **Autocrine**—hormone molecule secreted by a cell affects the secreting cell
APOPTOSIS

Intrinsic Pathway

DNA-Damaging Agent

p53

p53 (TP53)

p53 Inhibitors

BID

Translocation of truncated BID (tBID) to mitochondrial membrane

BCL2

IBID

BAK1

BAX

Cytochrome C

PAFAF1

Pro caspase 9

Apoptosome

Extrinsic Pathway

Adjacent Cellular Membrane

Extracellular Space

Cellular Membrane

BH3 interacting-domain death agonist

Pro caspase 8

Caspase 8

Pro caspase 3

Caspase 3

Caspase Cascade

Apoptosis
Invasion Metastasis Cascade
Cancer Cell Metabolism

Motility

Monocyte/macrophage

LACTATE

Effect T cell

Treg

Glucose

GLUT

IL-17A

Effector/memory T cell

Monocyte/macrophage

DC

Viability and cytokine secretion of immune cells

Motility

H+-pH down

MCT

LACTATE

Tumorsuppressor-/oncogenes

Chronic inflammation

NFKB

Effect T cell

LACTATE

Glucose-6-P

PEP-mediates phosphor transfer to PGAM

Glutaminolysis

Glycolsis

Pyrivate

Citrate

TCA

LDH-A

NADH

H+ down

H+-MCT

ROS scavenging

Radioresistance

Angiogenesis

IL-8

VEGF

EC migration

NF-kB

EC

TAF

CD 44

β1-integrins

Tumor cell migration

Metastasis

Patient survival

© 2011 American Association for Cancer Research

Cancer Research Reviews
Jak/Stat Signalling

1. ligand binding
2. receptor dimerization activates JAK phosphorylation of receptor
3. STAT binds phosphorylated receptor
4. JAK phosphorylates STAT
5. STAT dimer forms
6. STAT dimer travels to nucleus
7. STAT dimer binds DNA and changes gene expression

activation of transcription
EGFR Signaling
Src Signaling

A. Intact mTOR signaling
   Normal cell
   RTKs → IRS-1/2 → PI3K → AKT → mTORC1, mTORC2 → S6K

B. Constitutive signaling
   Tumor cell
   RTKs → IRS1/2 → PI3K → AKT → mTORC1, mTORC2 → S6K

C. Rapamycin-treated
   Tumor cell
   RTKs → IRS1/2 → PI3K → AKT → mTORC1, mTORC2 → S6K
   Rapamycin blocks mTORC1

D. Rapamycin + dasatinib
   treated tumor cell
   RTKs → IRS1/2 → PI3K → AKT → mTORC1, mTORC2 → S6K
   Rapamycin blocks mTORC1
   Dasatinib inhibits Src family kinase
Diagram 1: Illustration of Indirect Immunohistochemistry and Immunofluorescence methods.
CRISPR/Cas9

- **Targeting sequence**
- **Scaffold**
- **gRNA**
- **Cas9**
- **gRNA-Cas9 complex**
- **Target binding**
- **Cleavage**
- **Target DNA**
- **PAM**
- **Double-strand break**
- **Non-homologous end joining (NHEJ)**
- **Indels of variable length**
- **Homology directed repair (HDR)**
- **Precise insertion or modification**
Immunotherapy for NSCLC

• Atezolizumab (aPD-L1), 2016 approval
  • metastatic NSCLC that has progressed during or after first-line chemotherapy with a platinum-based drug.
  • 12.6 vs. 9.7 (docetaxel)
  • Side effects were less frequent

• Pembrolizumab (aPD-L1), 2016 approval
  • the first-line treatment in PD-L1 expressing metastatic NSCLC
  • second-line treatment of metastatic NSCLC (PD-L1 1%+)
  • 10.4 months and 12.7 months vs. 8.5 (docetaxel group)

• Nivolumab (aPD-1,) 2015 approval
  • second-line treatment of NSCLC
A SUMMARY OF DNA REPLICATION

1. Helicases unwind the parental double helix.
2. Single-strand binding proteins stabilize the unwound parental DNA.
3. The leading strand is synthesized continuously in the 5' → 3' direction by DNA polymerase.
4. The lagging strand is synthesized discontinuously. Primase synthesizes a short RNA primer, which is extended by DNA polymerase to form an Okazaki fragment.
5. After the RNA primer is replaced by DNA (by another DNA polymerase, not shown), DNA ligase joins the Okazaki fragment to the growing strand.

Overall direction of replication
The diagram illustrates the process of transcription, which is the first step in gene expression, leading to the production of RNA molecules. The process can be divided into four main stages:

1. **Binding** of RNA polymerase to the DNA at the promoter region, followed by local DNA unwinding.
2. **Initiation** of RNA synthesis with the addition of NTPs (Nucleoside Triphosphates) to the growing RNA strand.
3. **Elongation** of the RNA strand continues with further addition of NTPs, along with the unwinding of the template strand.
4. **Termination** occurs when a termination signal is reached, leading to the release of the RNA transcript and the disassembly of the transcription complex.

The DNA molecule acts as a template, with the coding strand being transcribed into an RNA transcript, which then undergoes further processing to become functional messenger RNA (mRNA).
Translation

1. A copy of a gene in the DNA is created. The copy, mRNA (messenger RNA), functions as a blueprint for a protein. Instead of thymine (T), uracil (U) is used in mRNA.

2. The mRNA and the two subunits of the ribosome are assembled.

3. Different tRNA (transport RNA) have different anticodons matching the various codons of mRNA. The amino acid, corresponding to the codon, is connected to the other end of the tRNA.

4. At the ribosome, tRNA associates with mRNA. The codon and the anticodon have to match, otherwise the tRNA falls off the ribosome.

5. A peptide bond is formed between the amino acids. The growing protein chain is moved to the tRNA in the right hand position. Then the ribosome moves one step along the mRNA-molecule in order for the next tRNA to bind.

6. The amino acid chain is folded to a protein.
NSCLC

Triple Negative Breast Cancer
Intracellular pathogen (e.g. Eimeria)
Cell-mediated Immunity

- B cell
- APC Antigen Presenting Cell
- Th0
- Naïve T-Cell
- Th1
- CTL
- IFN-γ
- IL-2
- IL-10
- Naïve T-Cell
- Th2
- B cell
- Ig
- Eosinophils
- Mast cell basophil

Extracellular pathogen
Humoral immunity (antibodies = Ig)
Regulatory T-Cells

- Inhibitory cytokines: Membrane-tethered TGFβ, IL-35, IL-10
- Cytolysis: Granzyme A or granzyme B, Perforin pore, Apoptotic effector T cell
- Metabolic disruption: cAMP, CD25, IL-2, Through gap junctions, Adenosine, Death due to cytokine deprivation
- Targeting dendritic cells: CTLA4, CD80/CD86, LAG3, MHC class II, IDO, Inhibition of DC maturation and function
### DISTRIBUTION OF Ig ISOTYPES

![Diagram of Ig isotypes distribution](image)

#### Table of Ig Isotypes

<table>
<thead>
<tr>
<th></th>
<th>IgM</th>
<th>IgG</th>
<th>IgA</th>
<th>IgE</th>
<th>IgD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heavy Chain</td>
<td>μ (mu)</td>
<td>γ (gamma)</td>
<td>α (alpha)</td>
<td>ε (epsilon)</td>
<td>δ (delta)</td>
</tr>
<tr>
<td>MW (Da)</td>
<td>900k</td>
<td>150k</td>
<td>385k</td>
<td>200k</td>
<td>180k</td>
</tr>
<tr>
<td>% of total antibody in serum</td>
<td>6%</td>
<td>80%</td>
<td>13%</td>
<td>0.002%</td>
<td>1%</td>
</tr>
<tr>
<td>Fixes complement</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Function</td>
<td>Primary response, fixes complement. Monomer serves as B-cell receptor</td>
<td>Main blood antibody, neutralizes toxins, opsonization</td>
<td>Secreted into mucus, tears, saliva</td>
<td>Antibody of allergy and anti-parasitic activity</td>
<td>B cell Receptor</td>
</tr>
</tbody>
</table>
B-Cells

A B-cell is triggered when it encounters its matching antigen.

The B-cell engulfs the antigen and digests it, then it displays antigen fragments bound to its unique MHC molecules.

This combination of antigen and MHC attracts the help of a mature matching T-cell.

Cytokines secreted by the T-cell help the B-cell to multiply and mature into antibody-producing plasma cells.

Released into the blood, antibodies lock onto matching antigens. The antigen-antibody complexes are then cleared by the complement cascade or by the liver and spleen.
Sandwich ELISA (enzyme-linked immunosorbent assay)
LegendPLEX Cytokine Profiling

**Principle of the Assay**

1. **Beads**
   - A4
   - A5
   - A6
   - A7
   - Sample

2. **Biotinylated Detection Ab**

3. Collect Data by Flow Cytometry

4. Quantitate Using LEGENDplex™ Software