Therapeutic Monoclonal Antibodies: An Overview

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Monoclonal Antibodies as Drugs

- Specificity of Monoclonal Antibodies fulfills Paul Ehrlich’s ideal of a “Magic Bullet”
- Presence of mouse protein sequence limited initial use of mouse MAbs due to “HAMA”
- Modern MAbs are recombinant proteins engineered to have little or no mouse sequence
- Therapeutic MAbs: Now largest class of recombinant therapeutic proteins
Outline of Topics

- Human IgG structure
- Effector functions of IgG molecules
- Methods for Engineering Recombinant Monoclonal Antibody (MAb)
- Advantages of therapeutic MAbs
- Limitations of MAb therapeutics
- Therapeutics for Autoimmunity and Immune Modulation
Overview (continued)

- Therapeutics for Transplantation
- Blood cancer Therapeutics
- Solid Tumor Therapeutics
- MAb for Eye Disease
- MAb for Heart Disease
- Anti-infective Monoclonal Antibodies

- Emerging Monoclonal Antibody Therapeutics
- Generic Monoclonal Antibodies?

(Please enlarge the presentation to full screen by clicking on the rectangular icon at the bottom of the screen)
Human IgG Secondary Structure

IgG
Human IgG Tertiary (3D) Structure

IgG ~145-160 kDa

[Diagram of IgG tertiary structure with labels for Fab and Fc regions]
Mammalian IgG Glycosylation

- Consensus carbohydrate structure on Fc of Mouse Monoclonal IgG:

- Reference: Rothman et al, 1989

\[\text{Gal-Sial-Man} \rightarrow \text{Man-Sial-Sial-Asn} \rightarrow \text{Sial-Man} \rightarrow \text{Fuc}\]

\[\text{Sial= N-Acetyl Glucosamine} \]
\[\text{Man= Mannose} \]
\[\text{Gal= Galactose} \]
\[\text{Fuc= Fucose} \]
\[\text{Asn= Asparagine (in protein)} \]
Functions of MAb Glycosylation

- Glycosylation necessary for maintaining MAb stability and half-life in the blood.
- Is required for secretion of the antibody by the antibody producing mammalian cells.
- Carbohydrates responsible for most of the micro-heterogeneity of a given MAb molecule.
Effector Functions: IgG Regions

- The **Fab** Regions are responsible for antigen recognition and binding.
- **Complementarity Determining Regions (CDRs)** are where the antigen actually binds.
- The **Fc** Region is responsible for MAb binding to Fc Receptors on Lymphocytes for Antibody Dependent Cellular Cytotoxicity (ADCC).
- **Fc** contains binding sites for Complement Fixation and activation.
- **Fc** carries most of the Carbohydrate.
Antibody Dependent Cellular Cytotoxicity (ADCC)

- ADCC depends on antibody (primarily IgG) bound to a cell.
- The bound antibody triggers Fc receptors (Type I, II, or III) on immune cells.
- Immune cells binding Fc may be lymphocytes, neutrophils, macrophages, or eosinophils.
- The triggered lymphocytes may lyse the target through extracellular lysis or through phagocytosis and intracellular killing.
Complement in blood is a complex system of protein factors that mediate lysis of a target cell after being triggered by the immune system.

Two IgGs that bind antigen and are within 20 nm of each other may bind complement C1q

Antibody bound C1q then activates the cascade of complement factors from blood.

Activation of the complement cascade may result in lysis of the target cell bound to antibody.
Monoclonal IgG Subclasses

- Most Therapeutic MAbs are IgGs, specifically IgG1 subclass.
- IgG1 fixes complement and also activates ADCC.
- IgG2 and IgG4 subclass MAbs have also been used but IgG2 do not activate ADCC as well.
- Fab fragments of IgG are also used in some therapeutics, but do not trigger ADCC or complement activation.
Antibody Fragments as Drugs

- The Fab or F(ab)2 sections of some MAbs have been developed as drugs.
- Fab has much smaller size (about one-seventh)
- Smaller size may cause much faster clearance from the blood and tissues.
- Smaller size means more rapid penetration of tumors or tissue
- Fab or F(ab)2 can be conjugated to PEG (e.g., Cimzia) for stability and increased half-life in blood.
- Fabs may be conjugated to toxin (for cancer drugs)
Antibody Genes

- An antibody is coded by a number of genes
- C(H) genes code for heavy chains constant regions
- C(L) genes code for light chains constant regions
- There are families of dozens of Variable (V) region genes providing diversity for recognizing different antigens.
- V region genes recombine with the C(H) or C(L) genes along with other genes, D (heavy chains) or J (both).
Antibody Genes

- Heavy chains: V(H) genes recombine with J genes and D Genes. V(H)DJ recombines with C(H) Gene
- Light chains: Vk or Vλ genes recombine with J genes.
- C(L) gene then recombines VkJ or VλJ genes
- Hypermutation in the recombined V genes occurs through somatic mutation, providing additional antibody diversity.
Chimeric, Humanized, and Fully Human MAbs—What are the differences?

- **Chimeric MAbs** have Human IgG Fc structure with mouse V sequences in the F(ab’2 sections.

- **Humanized MAbs** have human IgG structure with some mouse amino acid sequences engineered into the CDRs for antigen recognition.

- **Fully Human MAbs** have all amino acid sequences derived from genes for human immunoglobulins.
Chimeric MAb Engineering
(Courtesy of Dr. Robert Segal, Abbott Diagnostics)

**Chimeric Antibody Engineering**

Mouse mAb

Mu-Hu Chimeric mAb

Transfer murine variable region genes into vectors appending human constant region genes

Chimeric mAb

*Antigen binding portion of murine mAb are transferred
Humanized Monoclonal Antibodies

- Human, Mouse, and Humanized MAbs
Methods of Producing Humanized MAbs

- Protein and molecular biology engineering methods used in conjunction with X-ray crystallography.
- Mouse Monoclonal antibody V region gene sequences identified and inserted into human antibody genes (plus some "framework sequences")
Engineering of Humanized MAbs

Steps:

1. Mouse Monoclonal Antibody identified with high binding constant.

2. Heavy and Light chain V sequences for the MAb Complimentary Determining Regions (CDRs) identified. “Framework” sequences also identified.

3. Human H and L chains engineered to have sequences for CDRs by in vitro mutagenesis and other techniques.

4. Engineered H + L genes expressed and secreted in appropriate mammalian cell lines.
Methods of Producing Human MAbs

For Human MAbs

- Phage Display: Libraries of human V + C genes screened; then the selected genes transfected into mammalian cells.
- “Humouse”: Monoclonal antibodies produced in mice transgenic for human IgG genes
**Phage Display Fusion Proteins**

**Bacteriophage Phage Display** is the most prevalent method of selecting recombinant antibodies.
Phage Display Method of deriving Fully Human MAbs

- Immune or non-immune antibody genes expressed in phage display libraries.
- Antibody portions on surface of phage viruses selected by "panning" with antigen.
- Selected phage subjected to cycles of panning to isolate of high affinity binders.
- Phage DNA for antibody transfected into mammalian cell line for expression of recombinant MAb.
DNA for human C(H), V, and kappa genes isolated
DNA transfected into mouse embryos that have kappa and mu genes “knocked out”.
This transgenic mouse is used for MAb production (next slide)
Transgenic Mouse Produces Fully Human MAbs

- Transgenic Mouse immunized with antigen
- Mouse spleen removed for splenocytes
- Splenocytes fused with Myeloma cells
- Hybridomas cloned in HAT medium and selected on antigen
- Cloned hybridomas secrete human IgG specific for antigen
Advantages of Human/Humanized/Chimeric MAbs

- MAbs have high specificity for their target and low cross-reactivity to normal human tissue.
- MAbs have a higher FDA approval success rate (18%) than New Chemical Entity drugs (11%).
- On binding antigen, MAbs may activate complement and immune cells’ Fc Receptors to increase potency.
- Serum half-life of intact MAbs are two weeks or more
Limitations of Therapeutic MAbs

- MAbs are large glycoproteins with less stability compared with small molecule drugs.
- They require refrigeration and I.V. or sub-cutaneous injection.
- There may be reactions against the antibody as a foreign protein (even if humanized or human) and sometimes antibody specific effects.
- There may be infusion or dermatological reactions.
- MAbs may be too potent in achieving effect, e.g. immune suppression by anti-TNF MAbs may cause opportunistic infections.
- High cost per treatment compared with small molecule therapy.
## MAbs for Autoimmune Disease

<table>
<thead>
<tr>
<th>MAb Name</th>
<th>Company</th>
<th>Indication</th>
<th>Target</th>
<th>Origin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Remicade infliximab</td>
<td>J &amp; J</td>
<td>Rheumatoid Arthritis, Crohn’s, Psoriasis, A.S.</td>
<td>TNF-α</td>
<td>Human</td>
</tr>
<tr>
<td>Humira adalimumab</td>
<td>Abbott</td>
<td>Rheumatoid Disease, Psoriasis, A.S</td>
<td>TNF-α</td>
<td>Human</td>
</tr>
<tr>
<td>Cimzia certolizumab</td>
<td>UCB</td>
<td>Crohn’s Phase III RA</td>
<td>TNF-α</td>
<td>Humanized Fab-PEG 40 kDa</td>
</tr>
<tr>
<td>Drug Name</td>
<td>Company</td>
<td>Target</td>
<td>Indication</td>
<td>Origin</td>
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</tr>
<tr>
<td>Simponi golimumab</td>
<td>J&amp;J</td>
<td>TNF-α</td>
<td>R.A./Psoriasis Ankylosing Spondilitis</td>
<td>Human</td>
</tr>
<tr>
<td>Xolair omalizumab</td>
<td>Genentech</td>
<td>IgE</td>
<td>Asthma</td>
<td>Humanized</td>
</tr>
<tr>
<td>Campath alemtuzumab</td>
<td>ILEX Oncology</td>
<td>CD52</td>
<td>Multiple Sclerosis (also CLL)</td>
<td>Human</td>
</tr>
<tr>
<td>Tysabri natalizumab</td>
<td>Biogen-Idec/Elan</td>
<td>α-4-Integrin</td>
<td>Multiple Sclerosis</td>
<td>Humanized</td>
</tr>
</tbody>
</table>
MAb Therapy for Autoimmunity

- Predominant therapeutic action is binding of TNF-α in tissues, blood, lymph.
- TNF activates immune cells and their accessory cells.
- Antibody neutralizes immune stimulation by TNF-α.
- For Treatment of Rheumatoid Arthritis, Psoriasis, Crohn’s Disease, Ankylosing Spondilitis and others.
Risks associated with TNF inhibitors

- MAbs against TNF-α pose a risk of infections due to pre-existing Tuberculosis or Hepatitis B.
- These MAbs also may increase risk of bacterial, protozoan or fungal infections.
- A slightly higher rate of malignancy has been shown for patients treated with some of these drugs.
Other mechanisms of action through binding of immune system targets such as:

- IgE for reduction of histamine release in asthma (Xolair)
- CD52 for inhibiting activation of T cells (Campath, also for CLL)
- Alpha-4 Integrin on T cells to block their binding to Vascular Cell Adhesion Molecule-1 in brain (Tysabri)
Xolair Binds to Human IgE

- Xolair MAb binds tightly to IgE, responsible for Asthma:
Xolair blocks binding of IgE to the high affinity IgE Receptor (Mast cells, basophils)

Xolair is indicated for patients > 12 years with Asthma inadequately controlled by inhalation steroids.

Xolair is not indicated for other allergy

Xolair is administered S.C. in physician’s office

Anaphylaxis may occur after administration and may recur
Tysabri for Multiple Sclerosis

- Tysabri is approved for treatment of M.S. as long as patients are monitored for Progressive Multifocal Leukoencephalopathy (PML)
- PML is a fatal brain infection that has been shown to occur in patients treated with Tysabri.
## MAbs for Transplant Rejection

<table>
<thead>
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<th>Target</th>
<th>Indication</th>
<th>Origin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zenapax (daclizumab)</td>
<td>Roche</td>
<td>CD 25</td>
<td>Kidney Rejection</td>
<td>Humanized</td>
</tr>
<tr>
<td>Simulect (basiliximab)</td>
<td>Novartis</td>
<td>CD 25</td>
<td>Kidney Rejection</td>
<td>Chimeric</td>
</tr>
<tr>
<td>Orthoclone OKT3</td>
<td>Ortho Biotech</td>
<td>CD3</td>
<td>Kidney Rejection</td>
<td>Murine</td>
</tr>
</tbody>
</table>
MAbs for Transplant Suppression
(Transplantation tolerance therapy)

- MAbs bind to and inhibit activation of T cells
- This is through binding of CD25 (Tac) or CD3
- CD 25 is the alpha chain of the IL-2 receptor
- T cell IL-2 receptors appear on activated T cells.
- They activate the immune response to graft tissue
- Reduction of T cell response to graft keeps it in a tolerant state.
MAbs for Transplant Rejection

- Zenapax, Simulect, or OKT3 are given in conjunction with standard transplant drug therapy.
- They are infused I.V. <24 hours before transplant.
- Infusions are then given every two weeks.
- Zenapax or Simulect decrease acute allograft incidence significantly compared with triple or double immunosuppressive therapy alone.
- Since OKT3 is murine IgG, there may be reactions to it as a foreign (mouse) protein.
# MAbs for Blood Cell Cancer

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<tbody>
<tr>
<td>Rituxan (rituximab)</td>
<td>Biogen-Idec/Genentech</td>
<td>CD20</td>
<td>Non-Hodgkin’s B cell Lymphoma (Also for Rheumatoid Arthritis)</td>
<td>Chimeric</td>
</tr>
<tr>
<td>Campath</td>
<td>LeukoSite Berlex</td>
<td>CD52</td>
<td>Chronic Lymphocytic Leukemia</td>
<td>Human</td>
</tr>
</tbody>
</table>
MAb Strategy for Blood Cell Cancer Therapy

- Blood Cells more accessible to MAb than solid tumor
- Cell surface antigen must be abundant, not shed, not internalized, and non-secreted.
- Cell surface antigen must be on all tumor cells
- Cell surface antigen must be necessary for tumor cell function
- CD20 is a calcium channel protein on B lymphocytes which meets these criteria.
- CD33 surface protein also meets criteria
CD20 B Cell Membrane Protein

- CD20 is found on pre-B cells and mature B cells but not on the plasma cell differentiated from B cells.
- Since plasma cells make free antibody, its synthesis is not blocked by anti-CD20 MAbs.
- CD20 is a transmembrane calcium channel protein.
- CD20 plays a role in B cell activation, proliferation, and differentiation.
- Treatment with anti-CD20 does not lead to immuno-suppression.
MAb Therapy for Lymphoma

- Response rates are higher when Rituxin is given in combination with CHOP chemotherapy.
- Response rate was 76% for the combination vs. 64% for CHOP alone.
- Anti-CD20 MAb therapy is now part of standard initial therapy for Non-Hodgkin’s B cell Lymphoma.
- Maintenance therapy with anti-CD20 MAb also increases time to regression, but not overall survival.
Radio labeled Immunoconjugates

Zevalin

Bexxar

Rituximab conjugate

Tositumomab conjugate
Drug-MAb Conjugate

Mylotarg (Calicheamycin-anti-CD33 MAb)
## Immunoconjugates for Blood Cell Cancer

<table>
<thead>
<tr>
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<th>Target</th>
<th>Indication</th>
<th>Origin</th>
</tr>
</thead>
</table>
| **Zevalin**  
 ibritumomab  
 Tiuxetan   | CTI              | CD20   | Lymphoma                    | Radiolabeled Cheleted Rituxin conjugate     |
|                   | Bayer            |        |                             |                                              |
|                   | Schering         |        |                             |                                              |
| **Bexxar**  
 tositumomab  
 +131I “ “   | Glaxo Smith-Kline | CD20   | Lymphoma                    | 131-I labeled Murine MAb + unlabeled       |
| **Mylotarg**  
 (gemtuzumab) | Wyeth            | CD33   | AML that is CD33 + and age > 60 yrs | Humanized IgG4 Coupled to Calicheamycin |
Advantages of MAb conjugates

- Conjugates to radioisotopes or Toxins have high killing efficiency when bound to tumor
- Relatively little MAb conjugate is required
- Nearby tumor cells also may be killed by radioimmunoconjugates even if MAb-conjugate is not bound to them.
- Anti-murine antibodies are rare
Disadvantages of MAb conjugates

- There are practical difficulties with administering radioisotope drugs to patients, who must be kept isolated in a hospital setting for several days.
- There are safety issues with storing and monitoring high energy radioisotopes.
- The radioisotopes have short half lives (for safety reasons).
- Drug may be released from drug-MAb conjugates into the body (Mylotarg) and it should not be used in conjunction with other chemotherapeutic agents.
Requirements for Radioimmunotherapy

- Exclusion criteria include: <15% bone marrow cellularity and low platelets and/or neutrophils.
- Other exclusion criteria are pregnancy and prior radiation therapy or stem cell transplant.
- For Zevalin, a dose of unlabeled Rituximab is given on the first day to saturate non-tumor CD20.
- For Zevalin, an initial trace indium-111 isotope conjugate is given for bioimaging the therapy (Yttrium is a beta emitter).
# MAbs for Solid Tumor Therapy

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<thead>
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</thead>
<tbody>
<tr>
<td>Herceptin trastuzumab</td>
<td>Genentech (Roche)</td>
<td>HER2/neu</td>
<td>Breast Cancer</td>
<td>Humanized</td>
</tr>
<tr>
<td>Avastin bevacizumab</td>
<td>Genentech (Roche)</td>
<td>VEGF</td>
<td>Colorectal, Lung, Breast cancer</td>
<td>Chimeric</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Glioblastoma</td>
<td></td>
</tr>
<tr>
<td>Erbitux cetuximab</td>
<td>Bristol-Meyers-Squibb</td>
<td>EGF Receptor</td>
<td>Colorectal Cancer</td>
<td>Chimeric</td>
</tr>
<tr>
<td>Vectibax panitumumab</td>
<td>Amgen</td>
<td>EGF Receptor</td>
<td>Colorectal Cancer</td>
<td>Human</td>
</tr>
</tbody>
</table>
Strategies for Solid Tumor MAbs

- Desirable targets: Abundant and not secreted
- Target receptor or antigen must be on all tumor cell surfaces
- Growth Factor receptors, e.g., HER2/neu, EGF
- ADCC and Complement fixation both may occur in MAb anti-tumor therapy
- Vascularization is a host property that may be blocked by anti-VEGF independent of tumor antigens.
MAb Target in Breast Tumor

**HER2/neu** is over-expressed in ~25% of breast cancers tested by molecular diagnostics.

**Herceptin** recognizes an extracellular domain of **HER2/neu**.

~15% of women with metastatic Br CA and over-expressed **HER2/neu** respond to **Herceptin**.

25% increased survival at 29 months with combined chemotherapy + **Herceptin**.
Epidermal Growth Factor Receptor

- EGF Receptor increases cell growth and has an autocrine mechanism.
- EGF Receptor is a target of MAb anti-tumor therapy.
MAb Targets in Colorectal Cancer

Epidermal Growth Factor (EGF) Receptor is over-expressed in many solid tumors:

- Colorectal cancer
- Non-small cell lung cancer
- Breast cancer
- Head and neck cancers

Binding of MAbs to EGF-R limits receptor activation and inhibits tumor growth
MAbs against EGF-R such as Erbitux or Vectibax limit tumor growth alone or in combination with Cisplatin.

Cures of established tumors have been observed in combination of anti-EGF-R MAbs with Cisplatin or doxorubicin.

Vectibax does not fully activate ADCC (IgG2 subclass).

Erbitux does activate ADCC.
Avastin blocks VEGF Binding to VEGF Receptor

MAb to VEGF blocks its binding to VEGF-R and therefore inhibits vascularization.

\[ +\text{VEGF} \rightarrow \text{vascularization} \]

\[ +\text{Anti-VEGF} \rightarrow \]
MAb Targets in Colorectal and other Cancers

- **VEGF** is Vascular Endothelial Growth Factor.
- **VEGF** is essential for tumor and normal tissue vascularization.
- **Avastin** is a MAb to **VEGF** that has shown efficacy in prolonging patient survival in colorectal, lung, Gliobastoma and breast cancers.
- **Avastin** is also in clinical trials for Ovarian cancer.
Macular Degeneration ("Wet" Form)

- Age related Macular Degeneration (AMD) consists of blood vessel leakage and vascularization in the retina that blocks vision.
Lucentis (Genentech, ranibizumab) has been approved for age related Macular Degeneration (ARMD)

Lucentis, an anti-VEGF Fab, blocks VEGF mediated vascularization in the Retina

Lucentis is delivered into the eye by intraocular injection.

In conjunction with photodynamic therapy, 25% of Lucentis treated patients showed a gain in visual acuity compared with either PDT alone or sham injection alone.
### MAb for Heart Disease

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</thead>
<tbody>
<tr>
<td>ReoPro</td>
<td>Elli Lilly</td>
<td>GPIIa/IIIb on Platelets</td>
<td>Angioplasty with or without Stent placement</td>
<td>Chimeric Fab Fragment</td>
</tr>
<tr>
<td>abciximab</td>
<td>Mfr. by J&amp;J</td>
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</table>
ReoPro Blocks Platelet Aggregation

- Reopro inhibits binding of aggregation factors to platelet GpIIa/IIIb, inhibiting clotting.
Abciximab (ReoPro) Anti-Thrombotic

- ReoPro is an Fab fragment that binds to the GPIIb/IIIa Receptor on platelets
- GPIIb/IIIa is required for clot formation
- ReoPro inhibits clot formation for up to 48 hours after administration
- ReoPro is approved as an anti-thrombotic agent before angioplasty with or without Stent placement
- ReoPro is not recommended for emergency surgery due to the increased risk of bleeding.
### MAbs for Infectious disease

<table>
<thead>
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<th>Indication</th>
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</tr>
</thead>
<tbody>
<tr>
<td>Synagis</td>
<td>Medimmune</td>
<td>Antigen on F protein on RSV viral coat</td>
<td>Respiratory Distress due to RSV</td>
<td>Humanized</td>
</tr>
<tr>
<td>Palivizumab</td>
<td>Medimmune</td>
<td></td>
<td></td>
<td>Humanized</td>
</tr>
<tr>
<td>Numax</td>
<td>Medimmune</td>
<td></td>
<td></td>
<td>Humanized</td>
</tr>
</tbody>
</table>
Emerging Therapeutic MAbs

- For Osteoporosis, **Denosumab** is a MAb directed to Osteoclasts. A BLA was filed with the FDA in 2008 (Amgen).
- Bispecific MAb Removab to CD3/Epcam for cancer; directs killer T cells to tumor (Trion).
- For Alzheimer’s Disease, an anti-beta Amyloid MAb, Bapineuzumab in phase III clinical trials (Wyeth/Elan).
Emerging Therapeutic MAbs (cont.)

- Anti-Anthrax MAbs for Inhalation Anthrax (ABthrax, Raxibacumab, Human Genome Sciences, BLA submitted)
- Monoclonal antibodies for Hepatitis B and Hepatitis C are in development
- Dozens more MAbs in clinical trials for cancer, autoimmune diseases, infections.
It may be possible duplicate mammalian glycosylation with genetically modified yeast.

Such yeast may be able to produce and secrete functional human monoclonal antibodies.

Monoclonal antibodies may be produced in mass in yeast culture at much less cost than mammalian cell culture.
References


Dr. Winkler, a former employee of Abbott, owns Abbott stock. He does not receive compensation from Abbott for this seminar, and it is not intended to promote Abbott products in this area.
If you would like a Certificate of Attendance, please also supply your name and address in the comments section.

Thank you for attending this seminar.

Martin A. Winkler, Ph.D.
Consultant