Harnessing evolution to make medicines
IgG mAbs are large (150,000 Da) Y-shaped protein molecules with two (H/L) chains. Associated VH/VL domains (=Fv at end of Fab arms) come together to form antigen binding site comprising a scaffold with six loops of variable sequence. Variability created by combinations of multiple genetic segments. Ab binds to infectious agent and can block infection, also can kill infectious agent by recruiting effector functions through Fc domains (stem).
Strategy of immune system

(1) random rearrangement (combination) of V-gene segments. (*Tonegawa 1976*); (2) surface display of antibody on B-cell; (3) antigen-driven selection; (4) secretion of soluble antibody from plasma cell; (5) affinity maturation.
### Best selling medicines

<table>
<thead>
<tr>
<th>BRAND</th>
<th>DISEASE</th>
<th>COMPANY</th>
<th>SALES ($bn)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Humira</td>
<td>rheumatoid arthritis</td>
<td>AbbVie</td>
<td>16.1</td>
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<tr>
<td>2. Harvoni</td>
<td>hepatitis C</td>
<td>Gilead</td>
<td>9.1</td>
</tr>
<tr>
<td>3. Enbrel</td>
<td>rheumatoid arthritis</td>
<td>Amgen/Pfizer</td>
<td>8.9</td>
</tr>
<tr>
<td>4. Rituxan</td>
<td>NHL</td>
<td>Roche/Biogen</td>
<td>8.6</td>
</tr>
<tr>
<td>5. Remicade</td>
<td>rheumatoid arthritis</td>
<td>J&amp;J/Merck</td>
<td>7.8</td>
</tr>
<tr>
<td>6. Revlimid</td>
<td>multiple myeloma</td>
<td>Celgene</td>
<td>7.0</td>
</tr>
<tr>
<td>7. Avastin</td>
<td>cancers</td>
<td>Roche</td>
<td>6.7</td>
</tr>
<tr>
<td>8. Herceptin</td>
<td>breast cancer</td>
<td>Roche</td>
<td>6.7</td>
</tr>
<tr>
<td>9. Lantus</td>
<td>diabetes (insulin)</td>
<td>Sanofi</td>
<td>6.0</td>
</tr>
<tr>
<td>10. Prevnar</td>
<td>pneumonia (vaccine)</td>
<td>Pfizer</td>
<td>5.7</td>
</tr>
</tbody>
</table>

Year 2016. Source: from genengnews.com. **antibodies red, chemicals black, others green**
Mouse-human therapeutic antibodies

Mouse monoclonal antibodies (mAbs) 1975

Humanized mAbs 1986
HER2 Herceptin 1998
VEGF Avastin 2004
PD-1 Keytruda 2014
PD-L1 Tecentriq 2016

Simple chimeric mAbs 1984
CD20 Rituxan 1996
EGFR Erbitux 2006

HER2 Herceptin 1998
VEGF Avastin 2004
PD-1 Keytruda 2014
PD-L1 Tecentriq 2016
Sequence conservation in V-genes

From hybridoma cDNA. (Orlandi 1989).
**Display of antibody fragment on phage**

**Phage vector.** VH/VL from anti-HEL D1.3 mAb. *(McCafferty 1990).*

**Phage ELISA**
Phage selection

Model selection: rare binders (scFv D1.3 to target HEL) isolated by multiple rounds of affinity selection. (*McCafferty 1990*).
Antibody libraries

10^6 clones from mouse immunized with phOx. \( K_d = 10 \text{ nM} \)

**immune mouse library**
(Clackson 1991)

10^7 clones from human donors, \( K_d = 10 \text{ µM} \)

**non-immune human library**
(Marks 1991, Griffiths 1993)

random combinatorial [Huse 1989]
Variation

**Mutator host**

100-fold increase $K_a$ ($K_d$ phOx 300 nM to 3 nM)

**Mutation in vivo.** (Low 1996)

**Chain shuffling in vitro.** (Marks 1992)
Selection stringency

**Low [Ag] & capture** *(Hawkins 1992)*

**“Monomeric display”** *(Bass 1990), (Hoogenboom 1991)*
Large synthetic libraries


**Binding specificities and affinities** from large primary synthetic Fab library >10^{10} clones. (Griffiths 1994)
**Human mAb templated by mouse mAb**

**Adalimumab (Humira).** Developed through Cambridge Antibody Technology and Knoll (BASF Pharma), later sold to Abbott. First human therapeutic antibody approved by US FDA for rheumatoid arthritis. For strategy see (Jespers 1994).
Phage antibody pharmaceuticals

**Growth factor:** PIGF, VEGF-2, GDF-8

**Chemokine:** CXCL13

**Ion Channel:** P2X4

**Receptor:** IL-21R, PSGL-1, TRAIL-R1, GM-CSFα2

**GPCR:** GLP1R, GIPr

**Cytokine:** IL-6, Blys, APRIL

**Protease inhibitor:** PAI-1

**Peptide:** Ghrelin, NKB, gp41

Human pharma target classes

<table>
<thead>
<tr>
<th>Phage antibodies on the market.</th>
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<tbody>
<tr>
<td>Adalimumab (TNF/Autoimmune)</td>
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<tr>
<td>Avelumab (PDL1/Cancer)</td>
</tr>
<tr>
<td>Belimumab (BAFF/Lupus)</td>
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<tr>
<td>Guselkumab (IL23/Psoriasis)</td>
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<tr>
<td>Necitumumab (EGFR/NSCLC)</td>
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<tr>
<td>Ramucirumab (VEGFR2/Cancer)</td>
</tr>
<tr>
<td>Raxibacumab (Anthrax)</td>
</tr>
<tr>
<td>Moxetumumab (CD22/HCL)</td>
</tr>
</tbody>
</table>

>60 antibodies from phage display have entered clinical trials; J. Osbourne, Medimmune