

Target Validation

For **two** of the targets from a list below provide a short description **in your own words** as to the most important observations that validate the development of therapeutics.

As an example, the development of penicillin analogs targeting the transpeptidase that makes the bacterial cell wall is “validated” by:

- observing that natural compounds that inhibit the enzyme have antibacterial activity,
- the lack of any such enzyme/pathway in the host (human),
- the essentiality of the cell wall to bacterial viability, and
- the fact that numerous other “agents” interfere with the bacterial cell wall integrity (e.g., lysozyme breaks it down, causing cell rupture due to inability to retain osmotic barrier).

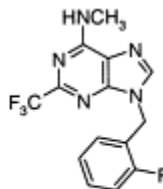
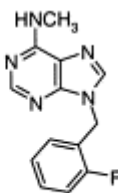
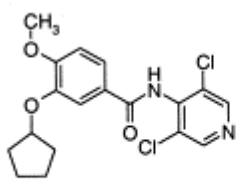
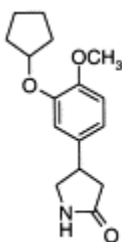
Provide the **earliest, specific and complete literature references** that provide or refute this validation hypothesis. Finally, indicate any limitations that are specific to this validation material. An example of an extensively developed validation for protein kallikrein 6 as a possible target for treatment of multiple sclerosis is shown on the next page.

Some examples of limitations are:

- in vitro* studies may not translate to *in vivo* results,
- the test conditions may not be relevant to human physiology
- the animal models used may not be relevant.

Target list:

- Depression: The following molecules serve as leads to develop potent antidepressant drugs via inhibition of phosphodiesterase 4. Provide information that validates phosphodiesterase 4 as an appropriate target for the treatment of depression.



- Alzheimer's disease is characterized by occurrence of plaques and intracellular tangles in the brain. The plaques are largely composed of insoluble aggregates of amyloid beta peptide ($A\beta$). There is no cure for the disease but many people think that the disease can be cured if formation of amyloid beta peptide aggregates can be avoided. Provide information that either validates or invalidates the amyloid beta peptide as a reasonable target for the treatment of Alzheimer's disease.
- Cancers in advanced stages are normally treated by combination of operations, ionizing radiation, and chemotherapy using drugs that kill dividing cells. Recently a new promising option—inhibition of angiogenesis—has emerged for treatment of many cancers. Avastatin (approved Feb 26, 2004) is the first of such compounds in the market. Provide information that validates Avastatin or related angiogenesis inhibitors as promising cancer treatment targets.
- Neuroregeneration. Immunosuppressive drug Tacrolimus suppresses the immune system via binding to a protein known as FKBP-12. This drug also enhances nerve growth after nerve injury. Provide data to show if FKBP-12 is a suitable target for finding neuroregenerative drugs.

Example of target validation: human Kallikrein 6 for Multiple Sclerosis (MS)
Partially adopted from a work of a student who took Chem162 last year

Summary:

Multiple sclerosis (MS) is characterized by progressive loss of myelin sheath around neurons. Histochemical studies performed in early 1960 demonstrated that proteins in myelin sheath are susceptible for trypsin-catalyzed degradation (1,2). In vitro degradation of myelin by trypsin and related serine proteases can be inhibited by protease inhibitors (3). Recently, a protein termed kallikrein 6 has been implied as a critical player in demyelination of nerve cells in MS patients (4)

Validating Studies:

A trypsin-like serine protease, myelencephalon-specific protease (MSP), was first discovered in the CNS of an adult rat by Scarisbrick et al. (5) and was found to have a human equivalent, human kallikrein 6 (hK6) (5,6). Serine proteases play an important role in the CNS including the regulation of neuronal migration during development, neurite outgrowth, synaptic plasticity, and neuronal degeneration and death. In healthy cells MSP contributes to the function of myelin-making cells by regulating oligodendroglia cells, which form part of the myelin sheath.

Scarisbrick first discovered MSP by isolating the protein from a rat using a PCR cloning strategy (5) and determined that MSP is a trypsin-like serine protease that is related to neuropsin and tissue kallikrein. It was discovered that MSP expression in the adult rat spinal cord was increased after an excitotoxic injury (5). In a separate study (7) it was observed that MSP was expressed in abundance in inflamed cells at sites of demyelination. Degradation of several myelin-associated proteins by MSP has been demonstrated (7,8). MSP/hK6 was examined in multiple sclerosis lesions of humans and animal models and is predominantly seen at sites where the immune cells are stripping the nerves of the protective proteins (7). Crystal structure analysis and biochemical studies suggest that hK6 is a trypsin-like degradative protein that is functional analog of rat MSP (8).

Potential Limitations:

- 1) While it appears that hK6 is involved in degradation of myelin sheath, it has not been proven that demyelination occurs mainly via the action of kallikrein 6. Present data do not rule out involvement of other proteases. If this is the case, specific inhibition of kallikrein 6 may not prevent demyelination.
- 2) The normal physiological role of kallikrein 6 is not well understood, but evidence suggests it is important for myelin-making cells. Thus, inhibition of kallikrein 6 may cause unwanted side-effects by altering the production of myelin.
- 3) The links between nerve injury, multiple sclerosis, and myelencephalon-specific protease levels is well established in studies using rat brains; such studies with kallikrein 6 in human brains are lacking.

References:

1. Wolman, M.; Hestrin-Lerner, S. A histochemical contribution to the study of molecular morphology of the myelin sheath. *J. Neurochem.*, **5**, 114-20 (1960).
2. Tuqan, N.A.; Adams C. W. M. Histochemistry of myelin. I. Proteins and lipid-protein complexes in the normal sheath. *J. Neurochem.*, **6**, 327-33 (1961).
3. Wood, J.G.; Dawson, R. M. C.; Hauser, H. Effect of proteolytic attack on the structure of CNS myelin membrane. *J. Neurochem.*, **22**, 637-643 (1974).
4. Bernett, M. J., Blaber, S. I., Scarisbrick, I.A, Crystal structure and biochemical characterization of human kallikrein 6 reveals that a trypsin-like kallikrein is expressed in the central nervous system, *J. Biol. Chem.*, **277**, 24562-24570 (2002).
5. Scarisbrick, I.A., et al., Nervous system-specific expression of a novel serine protease: Regulation in the adult rat spinal cord by excitotoxic injury, *J. Neurosci.* **17**, 8156-8168 (1997).
6. Scarisbrick, I.A., et al., MSP, a Trypsin-like serine protease, is abundantly expressed in the human nervous system, *J. Compar. Neuro.*, **431**, 347-361 (2001).
7. Scarisbrick, I.A., et al., Activity of a newly identified serine protease in CNS demyelination, *Brain*, **125**, 1283-1296 (2002).
8. Blaber, S. I., et al., Enzymatic properties of rat myelencephalon-specific protease, *Biochemistry*, **41**, 1165-1173 (2002).