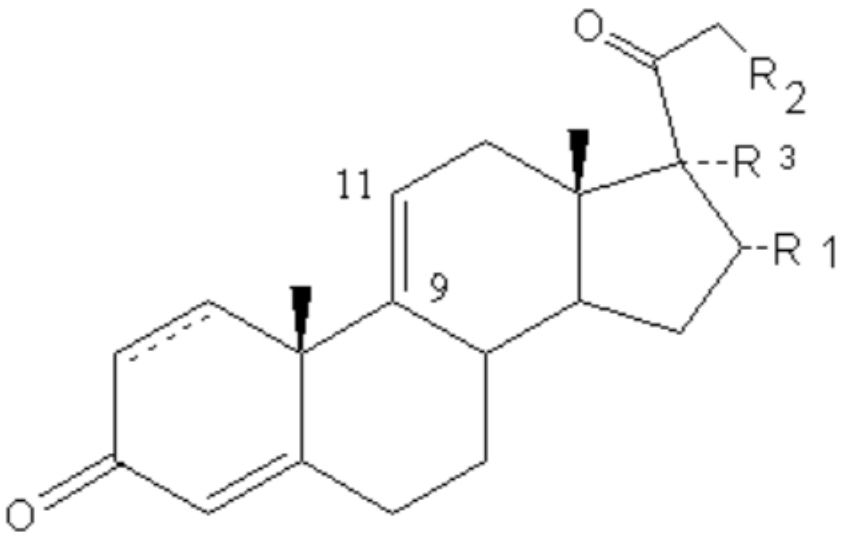
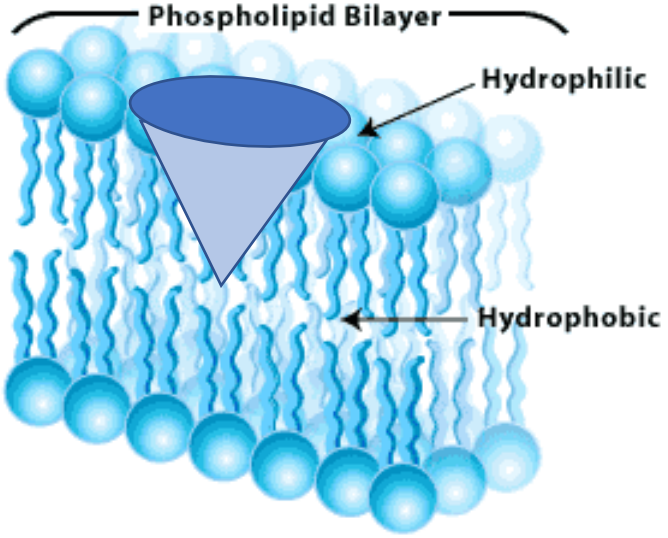
Delta 9,11 Steroids

The Glucocorticoid Challenge:

Dissociate anti-inflammatory and cell protection effects from limiting glucocorticoid side effects.



Steroid Mechanism Components



- Transrepression good: Steroid-receptor complex Inhibits NFκB and is anti-inflammatory. Upregulates anti-inflammatory proteins, down regulates expression of pro-inflammatory proteins.

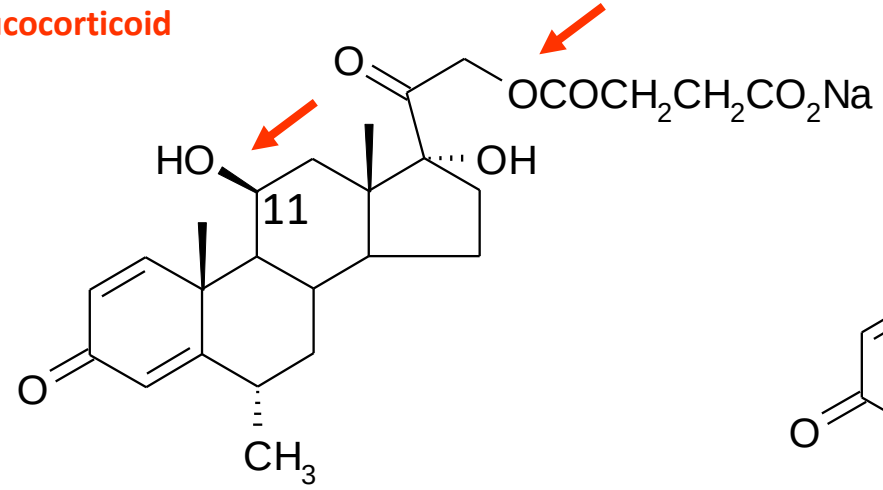
Transactivation bad: Steroids like prednisolone form a receptor-steroid dimer that binds to GRE (glucocorticoid response element) in the promoter region of target genes, thus regulating gene expression.

  - Stimulates gluconeogenesis (generation of glucose) and Inhibits glucose uptake in muscle and adipose tissue
  - Weight gain while stunting normal growth
  - Cushingoid appearance
  - Adrenal suppression
  - Bone fragility
  - Muscle wasting
  - Cardiac fibrosis

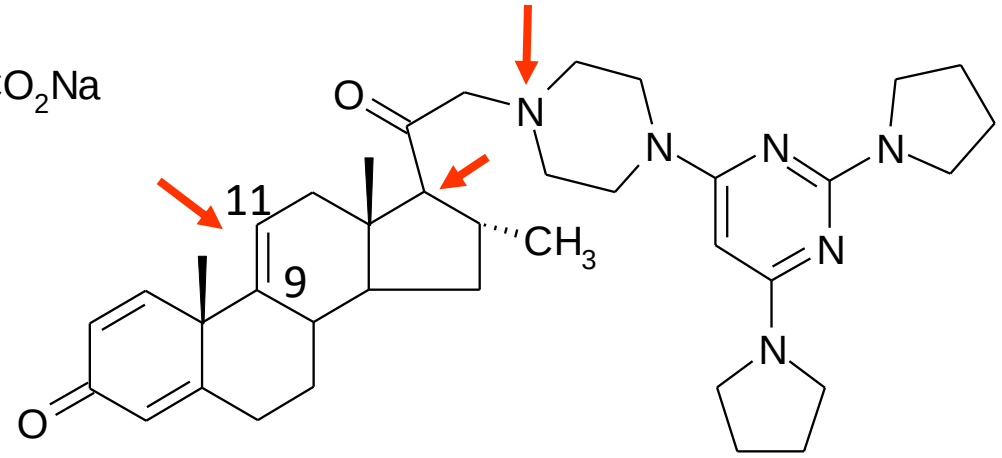
- Physical effect on membranes favored by delta 9,11 steroids over 11-OH or keto. D-ring substituents orient toward phospholipid head groups (lower permeability) and disorder the lipophilic core (poor lipid peroxidation chain reaction

Tirilazad and Methyl Prednisolone
The Upjohn Lazaroid Project

Glucocorticoid



Methyl Prednisolone Sodium Succinate



Tirilazad (PNU-74006F)

Key insights:

- 11-alpha hydroxy or delta 9,11 steroids lack glucocorticoid side effects
- Physical effects on membranes can be cytoprotective

Tirilazad Mesylate

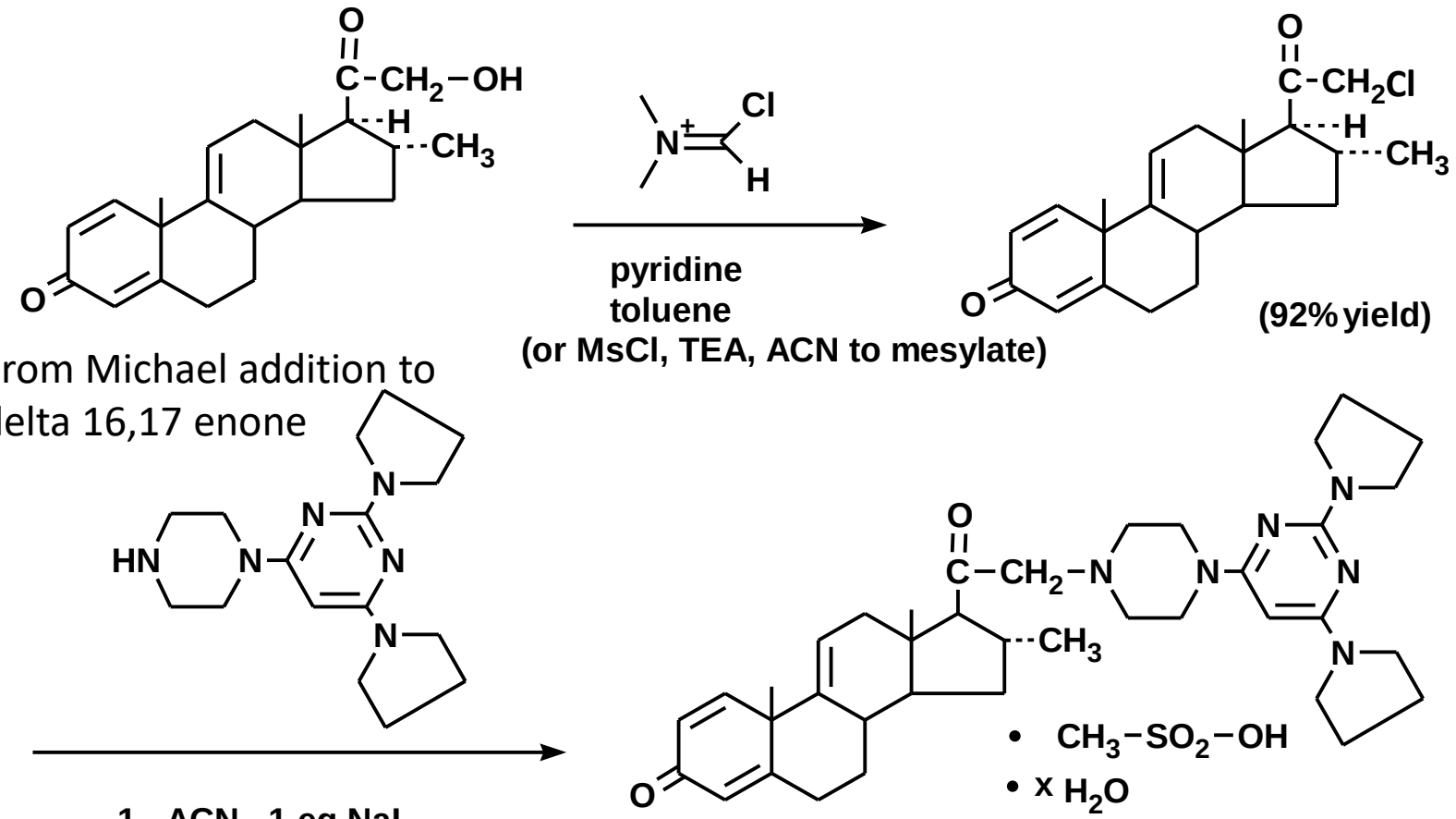
Membrane stabilizer, antioxidant, weak NFkB inhibitor

- Delta 9,11 steroids are better at physical effects on membranes than typical glucocorticoids that have an oxygen at position 11.
- Delta 9,11 steroid: lacks activational glucocorticoid effects

Evaluated clinically in head injury and subarachnoid hemorhage

Retrospective view: most membrane protective activities of tirilizad explained by physical effects on vascular cell membranes and probably NFkB inhibition

Synthesis of Tirilazad Mesylate



Wuts, P.G.M., Cabaj, J.E., Maisto, K.D., Synth. Commun., 1993, 23, 2199.

What did we learn?

Delta 9,11 steroids lack transactivation effects

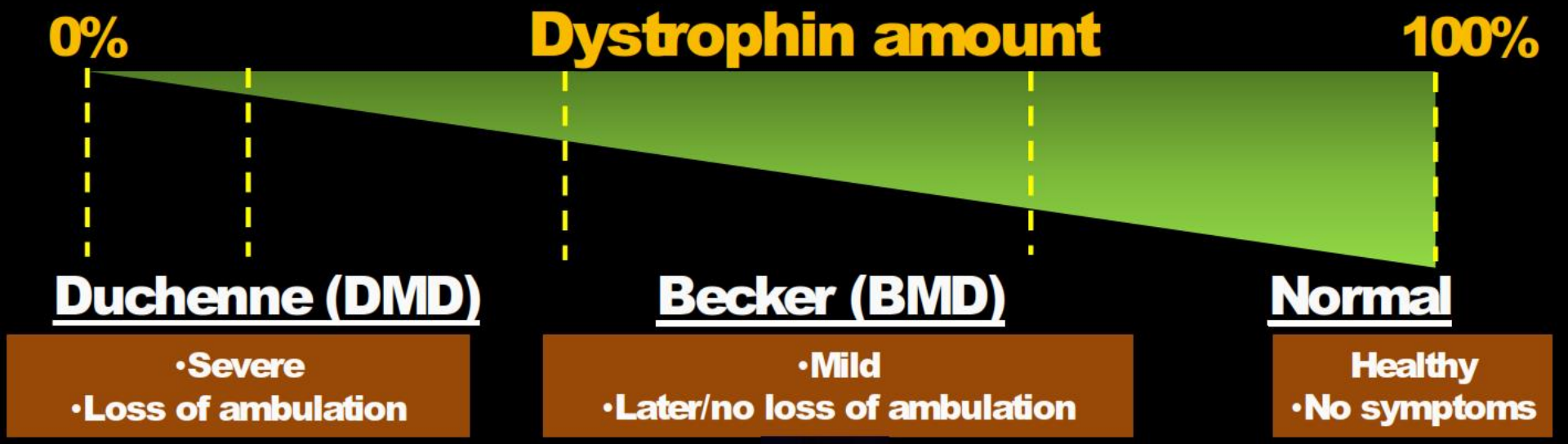
Delta 9,11 steroids are better at physical effects on membranes than typical glucocorticoids that have an oxygen at position 11.

And the synthetic methodology

Acknowledgments Lazaroids (Partial)

- Medicinal Chemistry
  - Gordon Bundy
  - John Palmer
  - Jon Jacobsen
  - Ruth TenBrink
  - Matthew Hester
  - Don Ayer
- Process Chemistry
  - Mark Krook
  - Mike Lipton
  - Tom Runge
  - Harold Karnes
  - Bruce Pearlman
  - A. Padilla
  - Peter Wuts
- Pharmacology
  - Ed Hall
  - Hartmut Jaeschke
  - Mark Braughler
- Physical Chemistry
  - Dennis Epps
  - Francois Kezdy
- Clinical
  - Eugene Means
  - Jack Luderer
  - Joe Fleishaker

Muscular Dystrophy - disease severity is related to dystrophin protein dosage



Duchenne (DMD)

- Severe
- Loss of ambulation

Becker (BMD)

- Mild
- Later/no loss of ambulation

Normal

Healthy

- No symptoms

Duchenne muscular dystrophy

- Chronically debilitating and life threatening
  - Onset early childhood (2-5 yrs), proximal muscle weakness
  - Dystrophic myopathy, progressive muscle weakness and wasting. Fibrosis. And complicated by steroid use
  - Loss of ambulation: ~10 yrs of age
  - Loss of abilities for aspects of daily living: late teens – 20's
  - Respiratory and cardiac failure unless ventilated

- Overview of the prevalence calculation
  - New born screening: 1/5,000 live born males
- Prednisolone's (standard of care)
- Prednisolone positive effects attributed to NFkB inhibition.

Chronic inflammation and corticosteroids

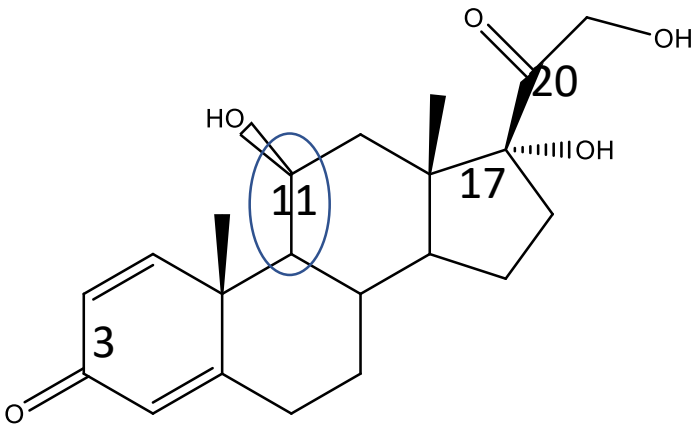
Steroids are potent anti-inflammatory drugs

- **Inhibition of NFkB pathways**

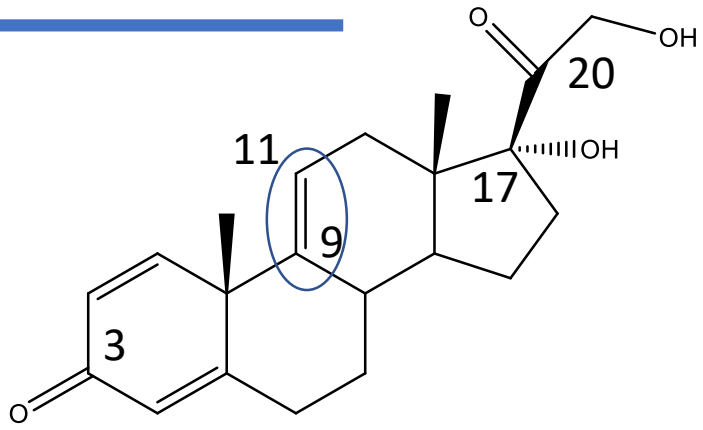
Glucocorticoids have multiple activities

- **Transactivation** (GRE-mediated positive gene transcriptional activity): side effects/ bad
- **Transrepression** (inhibitory transcriptional activity) – inhibit NFkB: efficacy / good
- **Physical effects on membranes**

Naïvity sometimes works for you…



Prednisolone

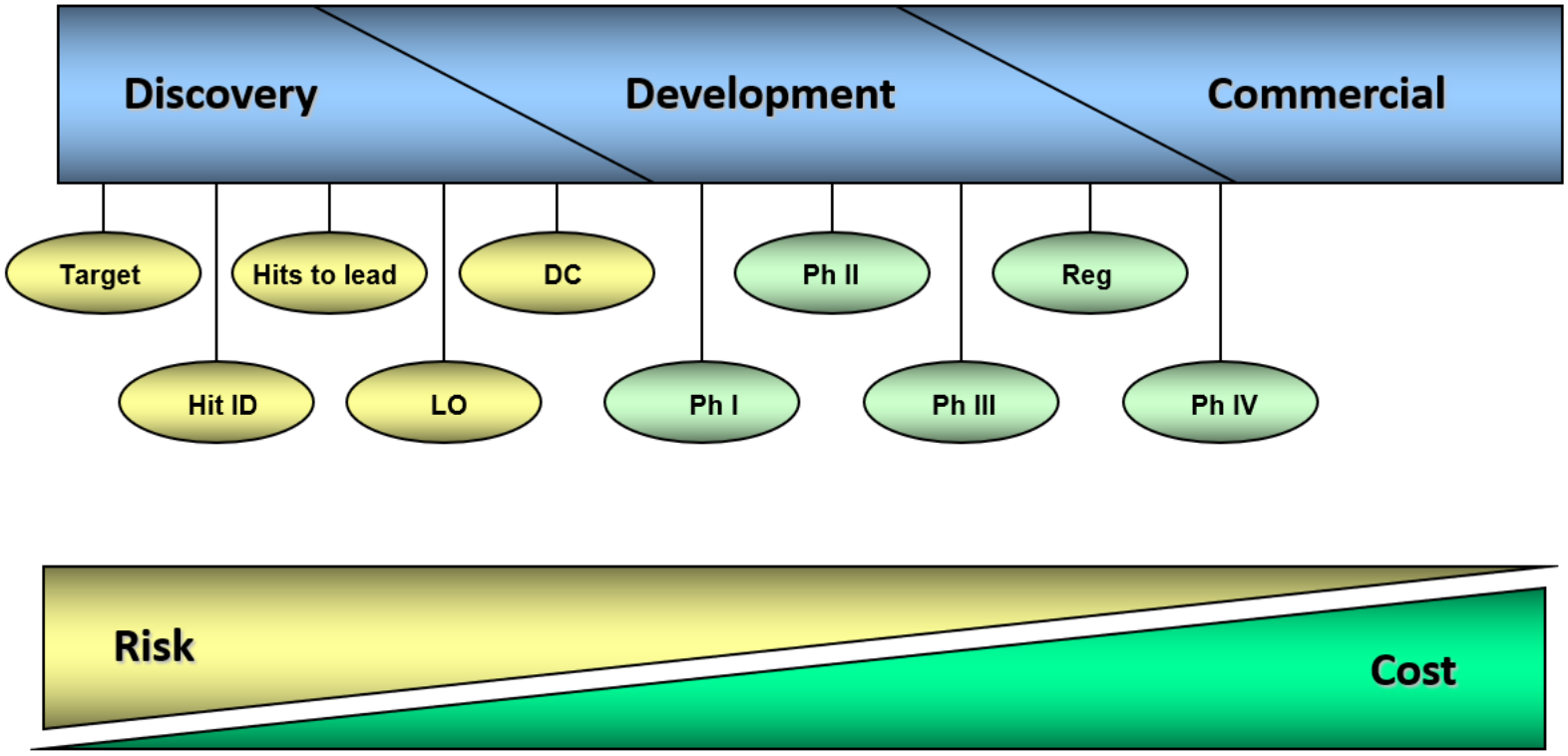


The non-glucocorticoid VBP-1 (assumed to be just a membrane stabilizer)

- Dystrophic mouse comparison trial: Designed as a test of membrane stabilization between similar molecules with different glucocorticoid activities.
- Result: VBP-1 equal to or superior to prednisolone in 6 month mouse trial
  - In retrospect, we learned that Prednisolone and VBP-1 are NFkB inhibitors with similar anti-inflammatory activity
  - VB-1 showed no glucocorticoid side effects and was a superior membrane stabilizer
  - Formed Reveragen to develop our discovery
- Delta 9,11 optimization became the next step : potency on NFkB, safer, better membrane stabilizer.

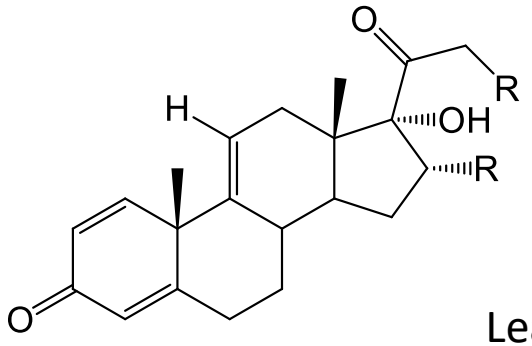
Drug Discovery and Development.

We parachuted in at Lead Optimization

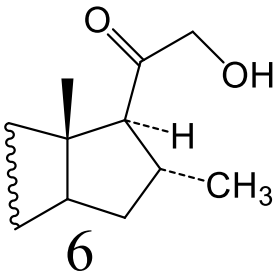


LO: optimize potency, safety, pharmacokinetics, in vivo efficacy

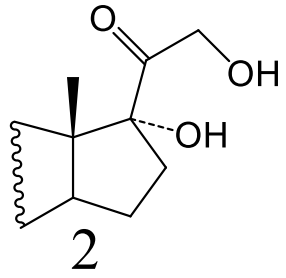
SAR NFkB



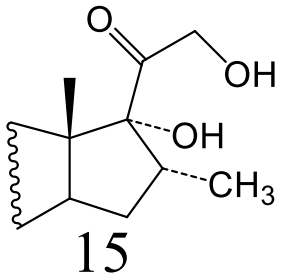
Most Potent



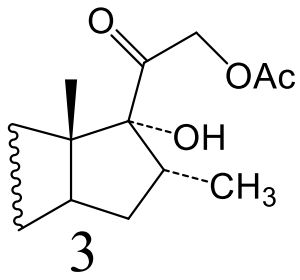
6



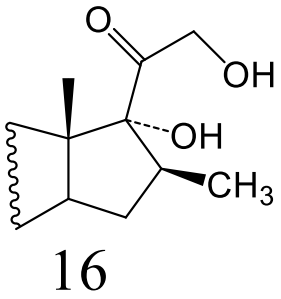
2



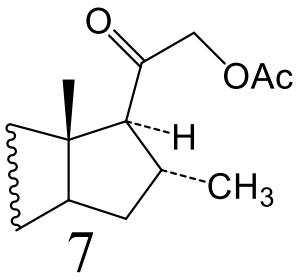
15



3

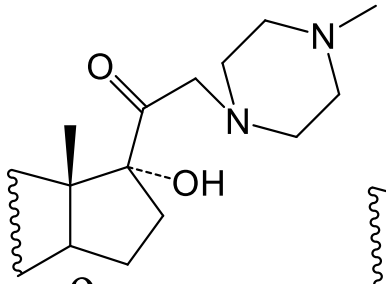


16

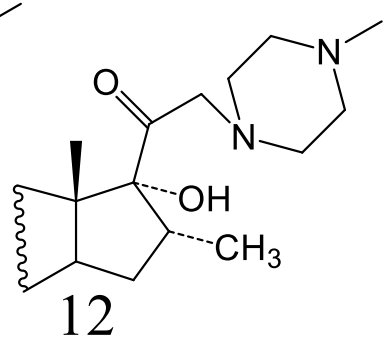


7

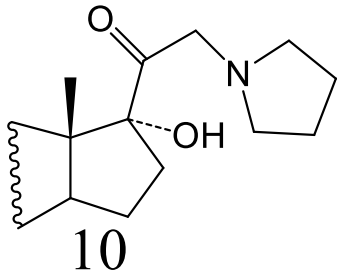
Least Potent



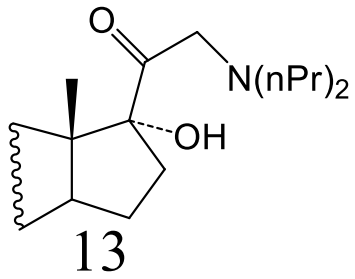
9



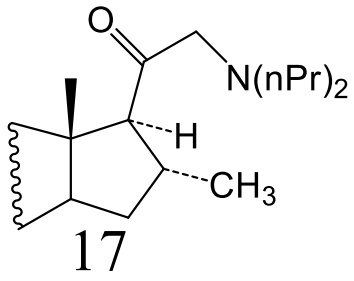
12



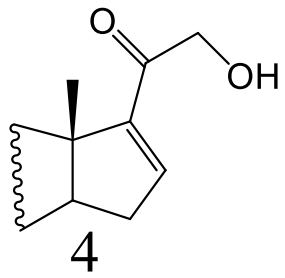
10



13



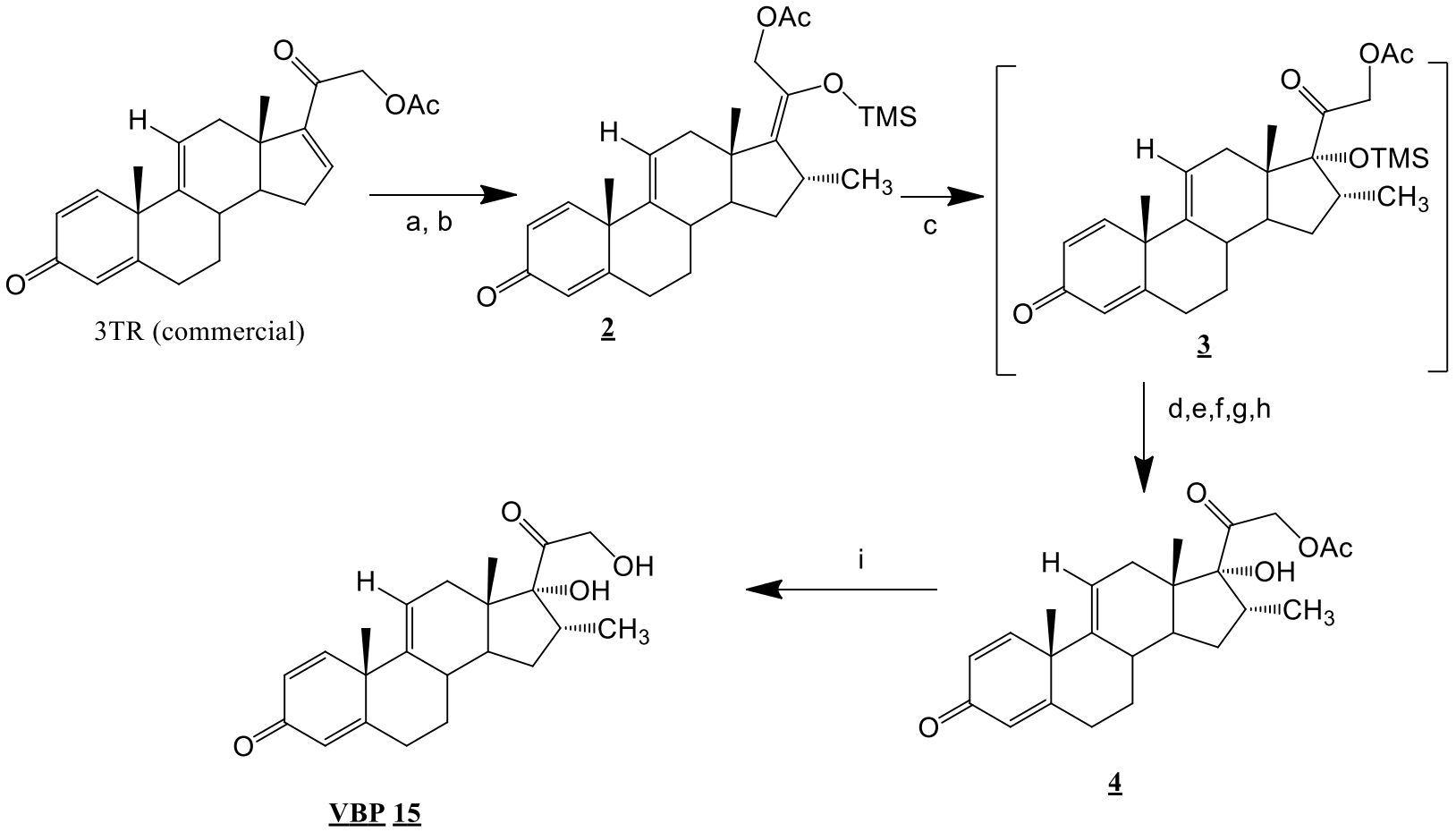
17



4

Credits: Bridge (Ed Hessler and Nancy Wicnienski): method and analog work

Vamorolone (VBP15) chosen from field of analogs for development

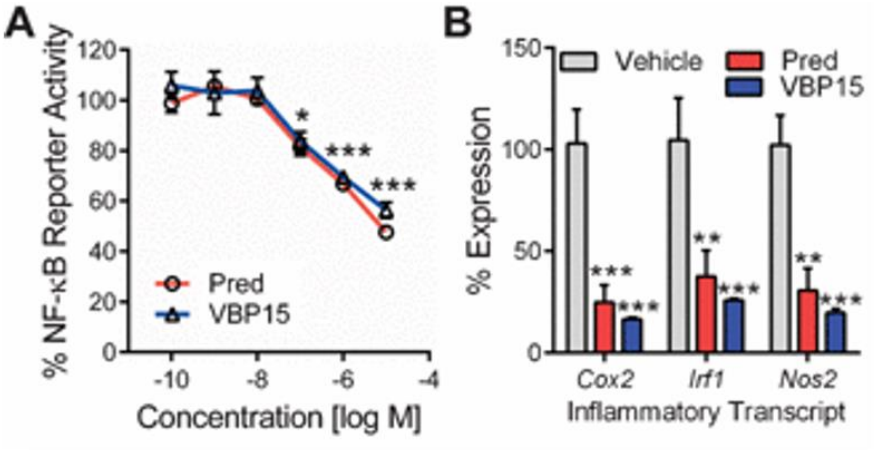


a)TMS imidazole, MeMgCl, THF; b) CuAc2, H2O, DMPU, MeMgCl, THF; c) peracetic acid, toluene, -10 degrees; d) NaHSO3, TFA; e) EtOAc, heptane; f) acetonitrile trituaration; g) HBr, CH2Cl2, 40 degrees; h) MeOH crystallization; i) K2CO3, MeOH followed by HCl and crystallization from MeOH/H2O

Credits: Bridge (Ed Hessler and Nancy Wicnienski), Ricerca, : method and analog work

Retains/Improves Transrepression

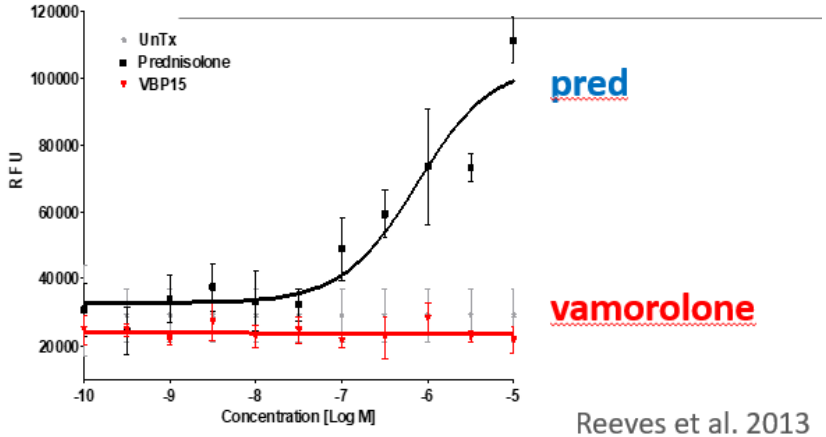
Vamorolone loses transactivation, retains transrepression and improves repair



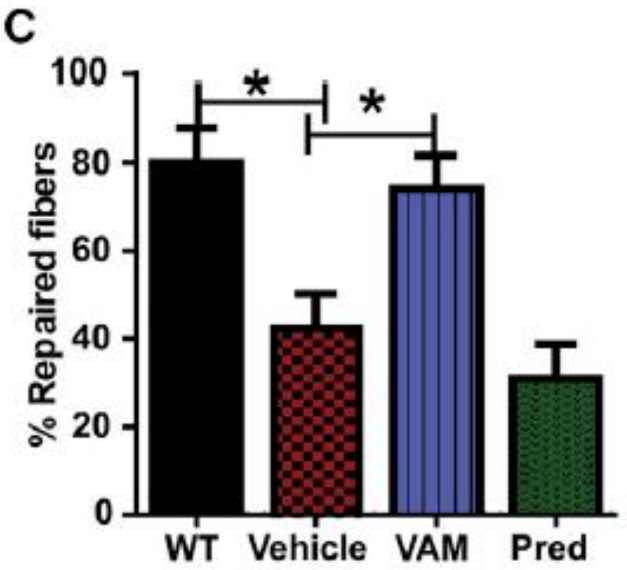
Vamorolone improves membrane repair

Prednisolone worsens repair

Loss of Transactivation



Reeves et al. 2013



RFU = fluorescence. The > the RFU, the more glucocorticoid side effects

Demonstrated activity and Safety

Mechanism

- NFkB Inhibition
- Membrane stabilization
- Repression but not activation

Animal models

- Duchenne muscular dystrophy
- Asthma
- Inflammatory bowel disease
- Arthritis
- Multiple sclerosis
- Glioma

Demonstrated safety in animals

- Loss of stunting of growth
- Loss of decrease in trabecular bone density
- Loss of cardiac injury
- Loss of problems with glucose homeostasis

Safety in phase 1 (84 volunteers)

- No changes in bone turnover, insulin resistance or adrenal suppression to 20 mg/kg

Phase 2a: 48 boys ages 4 to 7 with Duchenne.

- Completed October 2017. Safe to 6 mg/kg, dose dependent efficacy
- Long term extension: now 18 months at 2 and 6 mg/kg. No Cushingoid features, no stunting of growth.

Phase 2b: 116 boys ages 4 to 7 initiated

Vamorolone clinical trials in DMD

VBP15-002
Phase 2a
PK/safety
2 wks drug

VBP15-003
Phase 2a
6 months

VBP15-LTE
Long term extension
2 years

Expanded
Access
Program

N=48
0.25, 0.75, 2.0, 6.0 mg/kg/day
1/3rd to 8x prednisone in DMD

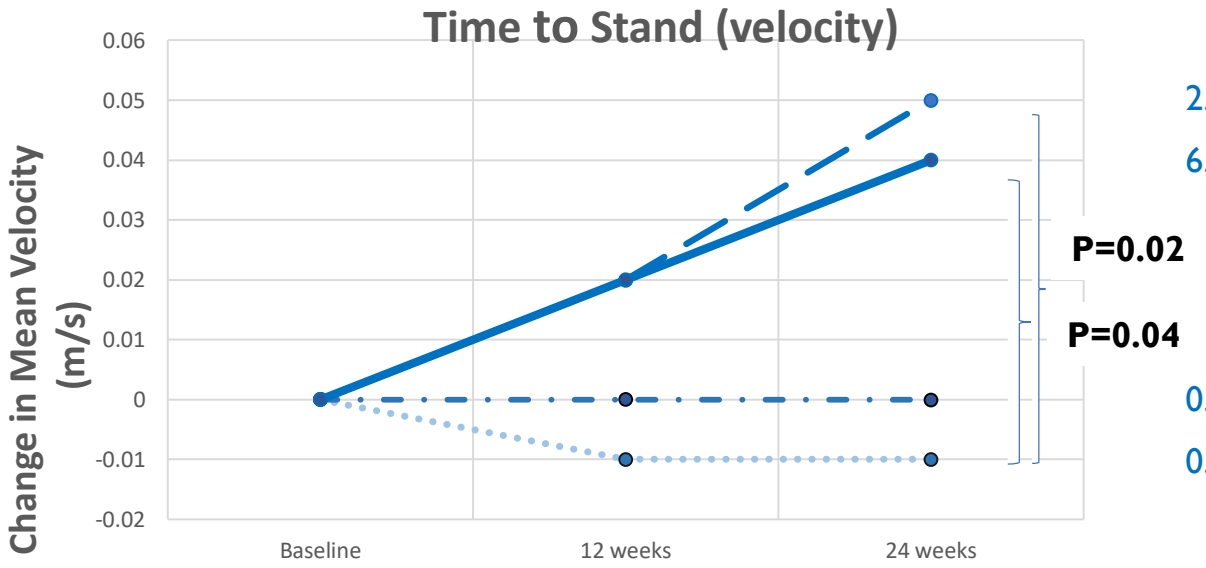
2.0, 6.0 mg/kg/day
3x - 8x prednisone in DMD

- **Phase 2a**
- **Open label**
- **N=48 4 to <7 years**
- **Steroid naïve**

- ❑ **Long-term extension: 45 of 48 remain on LTE**
- ❑ **At end of study =** 2.5 years treatment; transition to **EAP** (initial patients now transferred)
- ❑ Families/physicians do not wish to transition to glucocorticoids (pred, deflazacort)

Phase 2a : 6 month safety and efficacy (4 dose groups, four to seven year old boys)..dose dependent efficacy

Primary clinical efficacy outcome
Time to stand from supine (floor)



2.0 mg/kg/day
6.0 mg/kg/day

High dose groups
Similar to prednisone
3x-9x prednisone dose

0.75 mg/kg/day
0.25 mg/kg/day

Low dose groups
Similar to steroid naïve
1/3–1x prednisone dose

- Improvements in gross motor function tests significant within trial (between dose groups)
- Significant in comparison to steroid naïve natural history comparator (CINRG DNHS)
- At 6 months, improvement similar in magnitude to corticosteroids; *18 month comparisons pending*

Vamorolone vs. corticosteroids

	Vamorolone	Prednisolone	Comment
Slows Disease Progression	Yes	Yes	Clinical and preclinical similar efficacy
NFKb Inhibitor	Yes	Yes	Similar potency
Glucocorticoid side effects	No / less	Yes	Parents preference
Mineralocorticoid	Antagonist	Agonist	Cardiac benefit w/Vam likely
Membrane stabilization	>>	<	Likely long term benefit on muscle loss
Rodent models	Active	Active	muscular dystrophy, rheumatoid arthritis, allergen induced asthma, MS, inflammatory bowel disorders
First in class	Yes	No	

Key Milestones

- Orphan Drug Designation, Fast-track designation for Duchenne
- 4 week and chronic mouse and dog glp safety complete
- GLP gene tox studies complete
- GMP campaigns successful
- Formulation (suspension) stable for > 2 years and palatable
- Phase 1 complete: safe, dissociated activity confirmed
- Phase 2a complete: safe, effective in 4-7 year old boys
- Phase 2b/P3 underway.
- Partnered with Idorsia and Santhera
- Raised >$50M in non-dilutive capital.

Vamorolone developed by ReveraGen BioPharma, a privately held company funded by government (USA, EU), non-profit foundations, and option agreement (Actelion, Idorsia, Santhera).

- ❑ 12 Foundations
- ❑ US NIH (NCATS, NINDS, NIAMS), EC Horizons 2020

Acknowledgements

- Reveragen Team: Jesse Damsker, Kanneboyina Nagaraju, Laurie Conklin, Suzanne Gaglianone, John van den Anker, Eric Hoffman, John McCall
- Clinical trial management: TRiNDS LLC and Camden group
- Financial support: US and EC government grants, 12 non-profits, Idorsia

- http://vision-dmd.info/

Subarachnoid Hemorrhage: Tirilazad Clinical Trials

- 41 centers, 1023 patients on V, Trz (.6, 2.0, 6.0 mg-kg-10 days)
- Safe and effective in female population
- Primary effect is increase in survival. 43% vs 21% in vehicle (p=.007). 28% decrease in symptomatic vasospasm (p=.046). Best in severe cases.
- Consistent gender differences due to PK
- Local venous irritation most common medical event.
- Registration in many European countries but not in the US.

Overview

- **Mechanisms of action (efficacy) – *in vitro***
  - NF-κB inhibition similar to prednisone
  - Inhibition of inflammatory transcript in splenocytes
  - Membrane stabilization
- **Measures of efficacy – *in vivo***
  - Muscle strength. Superior to prednisolone
- **Measures of pharmacological safety – *in vitro***
  - Adrenal suppression reduced
  - GRE-mediated hormonal toxicity reduced
- **Measures of pharmacological safety – *in vivo***
  - Stunting of bone growth avoided
  - Immunosuppression reduced
  - Muscle and heart myocyte degeneration and fibrosis reduced
- **Summary of mode of action**
  - Prednisone without GRE activation