

Mitigation and Treatment of Radiation Injuries via Antagonism of the Renin-Angiotensin System

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NCI: mechanisms of chronic radiation injury

NIAID: prevention and treatment of chronic radiation injury

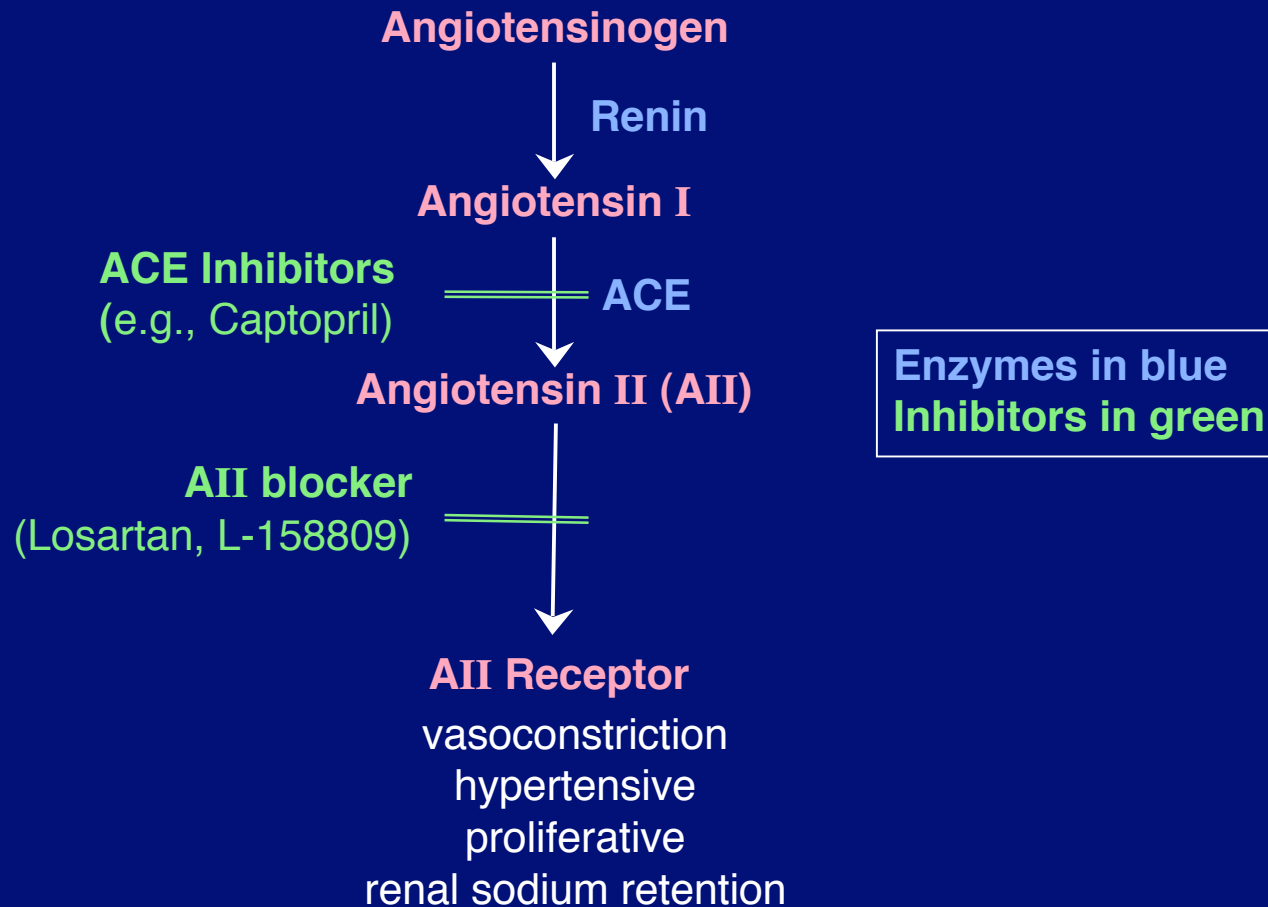
The Renin-Angiotensin System and Radiation Injury

- Evidence has been mounting since the late 1980's that antagonism of the renin-angiotensin system (RAS) is effective against some radiation injuries.
 - Efficacy seems limited to chronic radiation injuries.
- Clinically, RAS antagonism is used to treat hypertension:
 - ACE inhibitors (e.g., captopril, enalapril, ramipril) to prevent production of angiotensin II (AII).
 - AII receptor blockers (e.g., losartan) to block the AII type 1 (AT₁) receptor.
- Their efficacy in radiation injury is not SOLELY due to direct anti-hypertensive action.
 - They are effective at non-hemodynamic doses.
 - Antihypertensives that do not directly antagonize the RAS do not work against radiation injury.
- RAS antagonism works in post-irradiation regimens.
 - It does not work in pre-irradiation regimens.

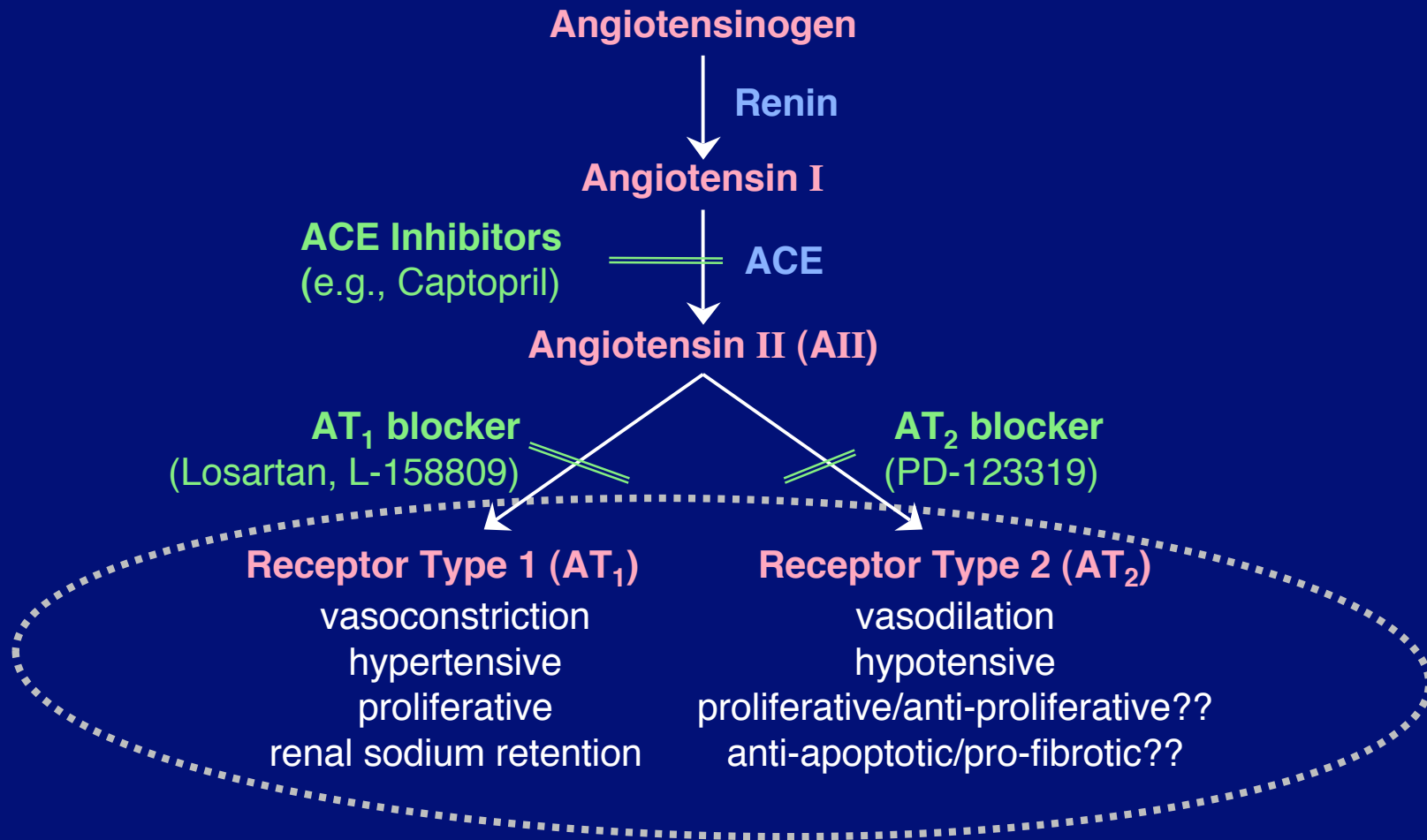
Suppression of the RAS and Radiation Injuries

Author	Drug	Schedule, endpoint and system
Robbins & Hopewell (1986)	Captopril (ACE inhibitor)	Mitigation of acute renal injury (pig)
Ward et al. (1988)	Captopril	Mitigation of pulmonary dysfunction (rat)
Ward et al. (1989)	Other ACE inhibitors	Mitigation of pulmonary dysfunction (rat)
Ward et al. (1990)	Captopril	Mitigation of acute and late skin damage (rat)
Cohen et al. (1992)	Captopril	Treatment of chronic renal injury (rat)
Moulder et al. (1993)	Captopril	Mitigation of chronic renal injury (rat)
Cohen et al. (1994)	Other ACE inhibitors	Mitigation and treatment of chronic renal injury (rat)
Moulder et al. (1996)	L-158809 (AT₁ blocker)	Mitigation and treatment of chronic renal injury (rat)
Cohen et al. (1996)	ACE inhibitors	Treatment of renal injury after BMT (human)
Molteni et al. (1997)	L-158809 (AT₁ blocker)	Mitigation of chronic lung injury (rat)
Cohen et al. (2002)	High dietary salt	Mitigation of chronic renal injury (rat)
Cohen et al. (2003)	Losartan (AT ₁ blocker)	Treatment of chronic renal injury (human)
Moulder et al. (2004)	PD-123319 (AT₂ blocker)	Mitigation of chronic renal injury (rat)

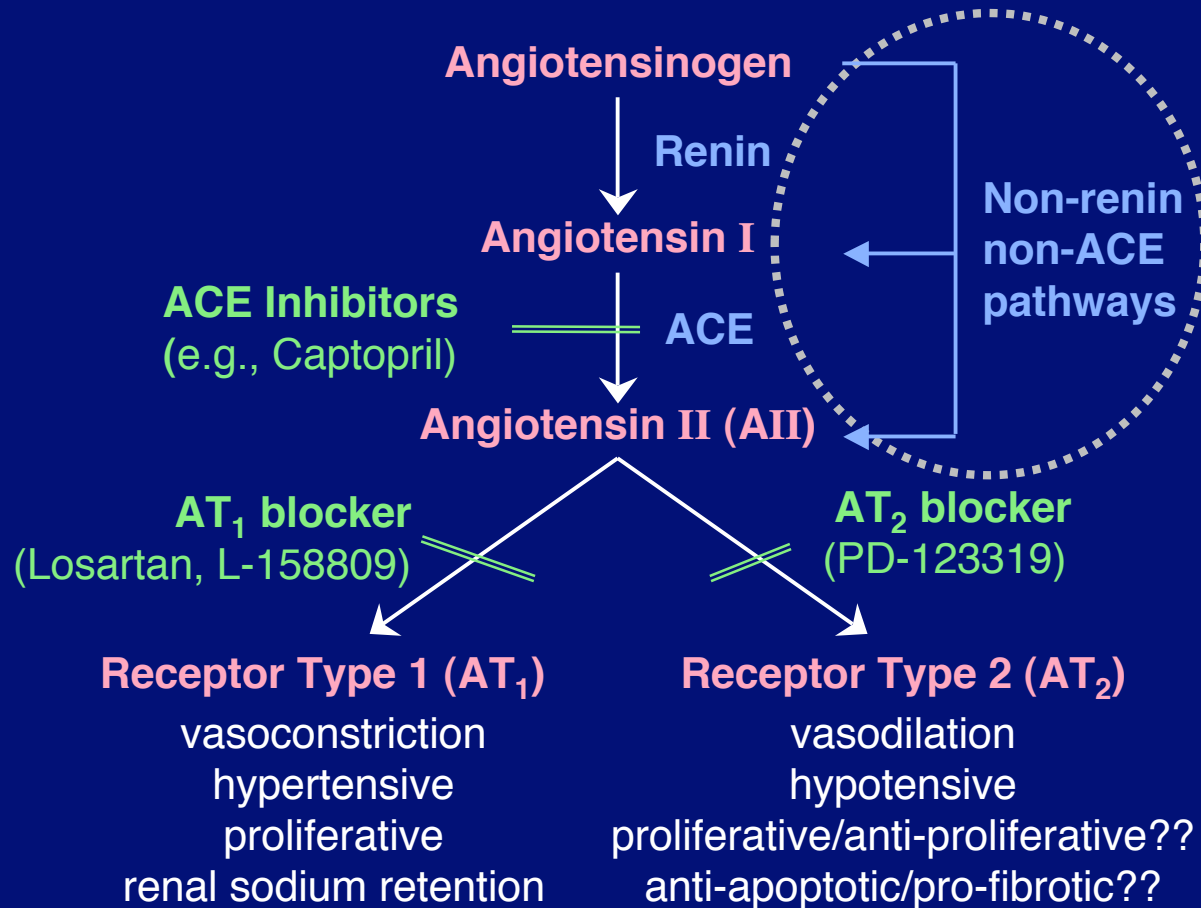
The Renin-Angiotensin System: the simple version



Reality is a Bit More Complex: there is more than one type of receptor

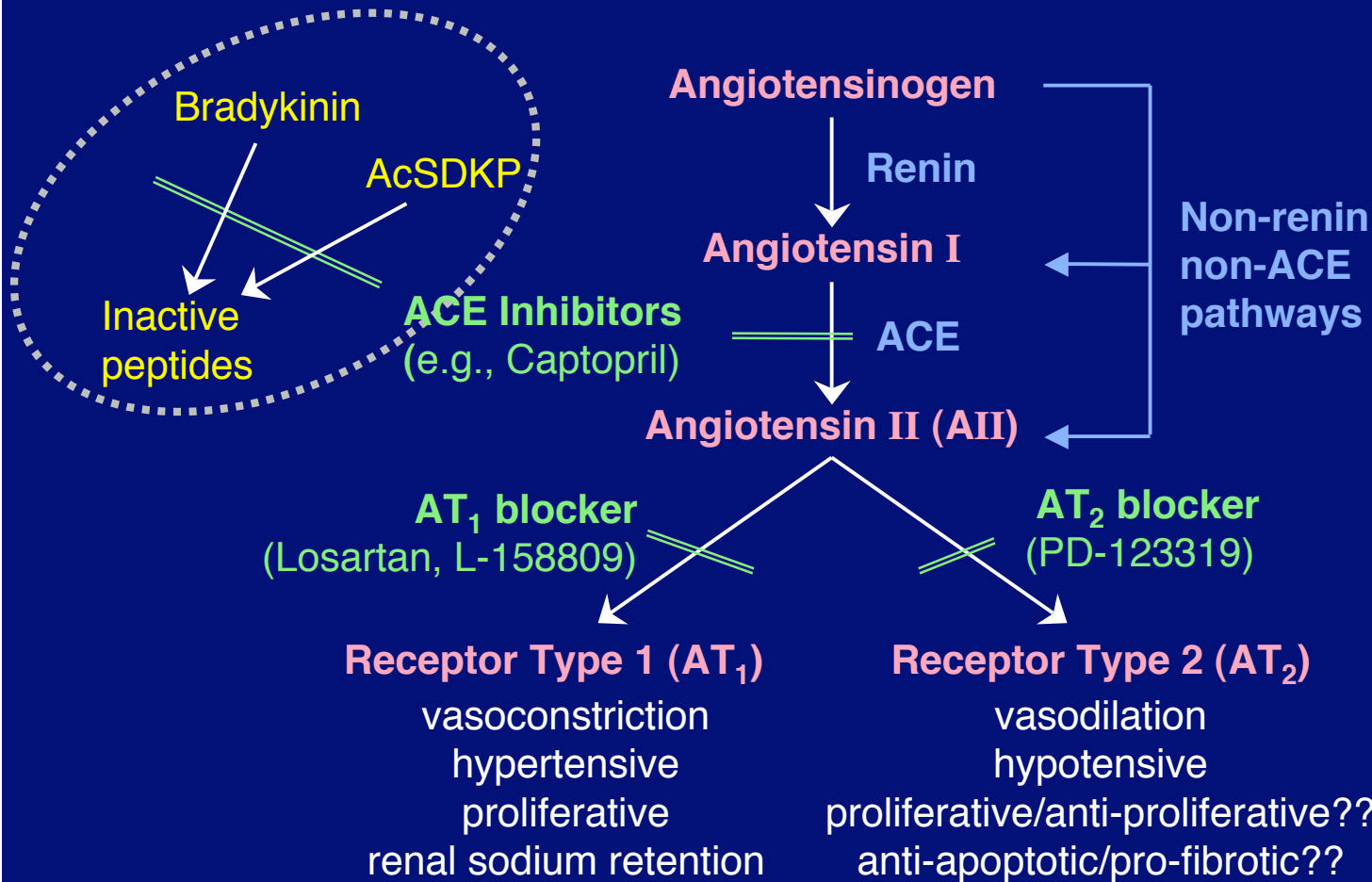


There are Other Pathways that Make AII



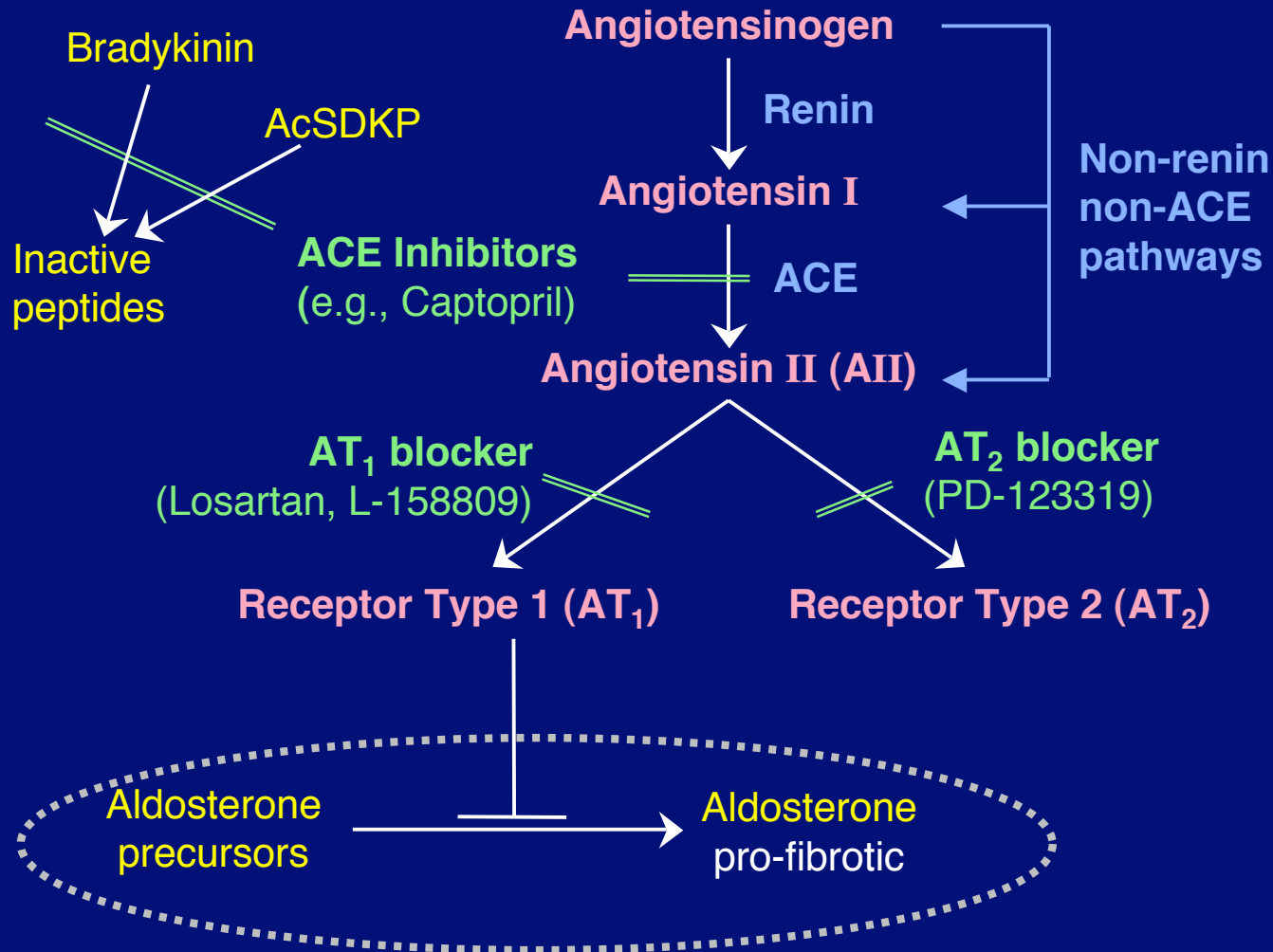
- **Thus ACE inhibitors do not eliminate all AII.**

ACE Has Other Actions

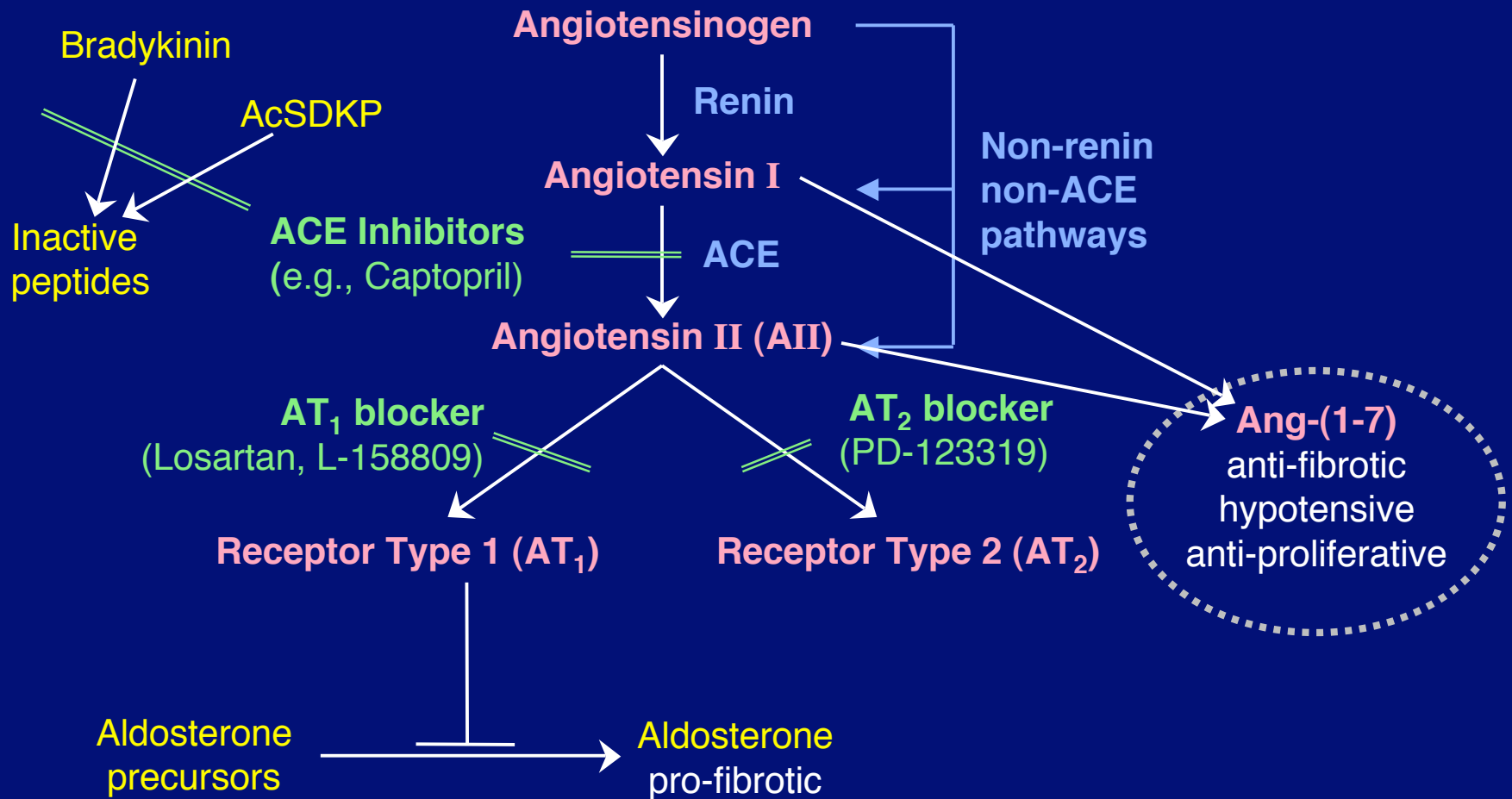


- Thus bradykinin and AcSDKP levels go up when ACE inhibitors are used.

There Might be an Aldosterone Connection



Some AI or AII Metabolites have Activity



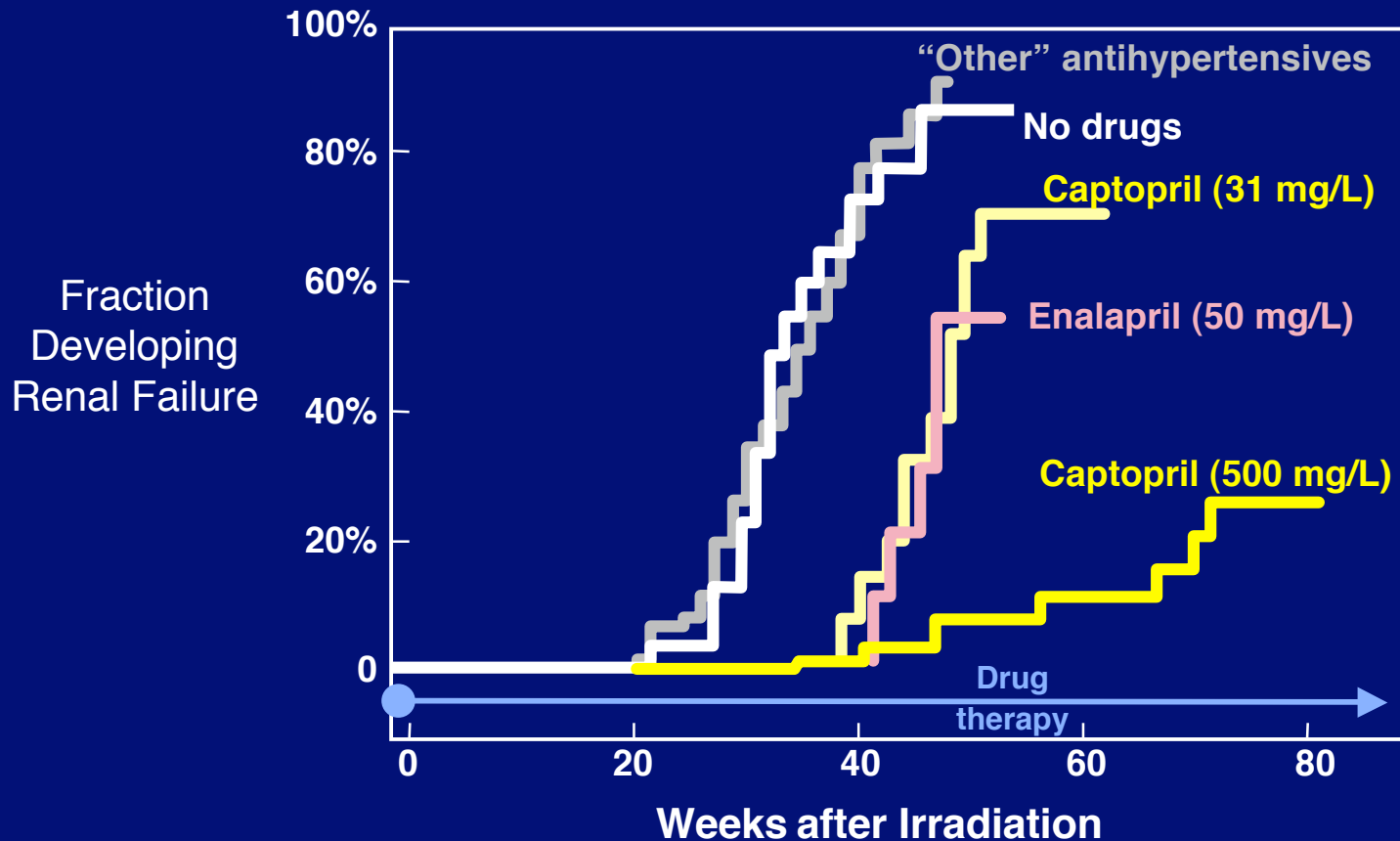
Preventing, Mitigating and Treating Radiation Injuries



- **NIH recommended the following terminology in 2001:**
 - prophylactic agents/protectors are given before radiation exposure;
 - mitigators are given after exposure, but before evidence of injury is apparent;
 - treatments are given after overt symptoms develop.
- **All three classes are of potential benefit in radiation oncology.**
- **But pre-irradiation approaches have very limited utility as countermeasures for nuclear or radiological terrorism.**
 - Pharmacologic protection of first responders would require an agent with essentially ZERO ACUTE TOXICITY.

Mitigation of Radiation Nephropathy

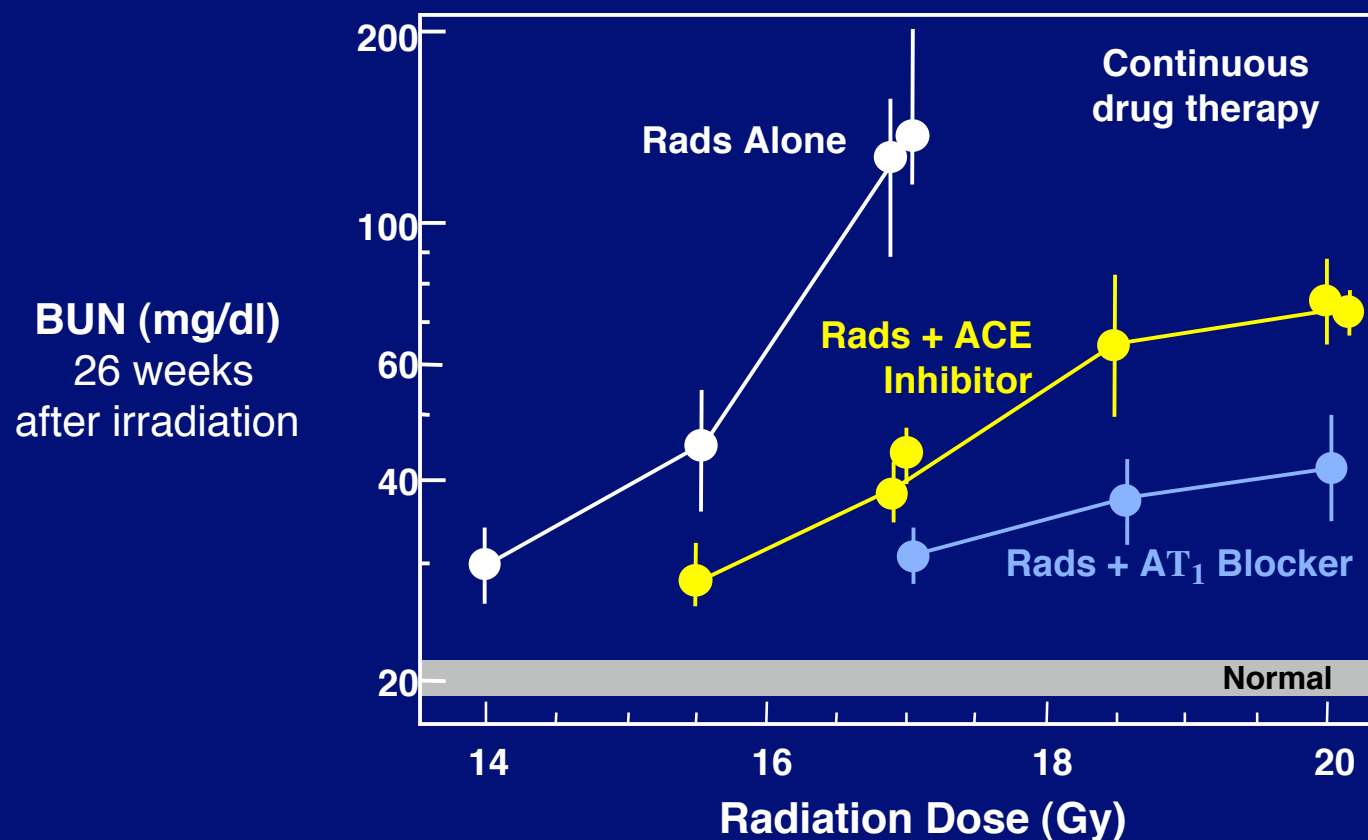
(Moulder et al., Cancer Treat Res, 1998)



- **ACE inhibitors are effective in mitigation of radiation nephropathy.**
 - Antihypertensives that do not antagonize the RAS are ineffective.
 - ACE inhibitors are effective even at doses that do not have anti-hypertensive activity.

Mitigation by AT₁ Blockade vs. ACE Inhibition

(Moulder et al., Rad Res, 1998)

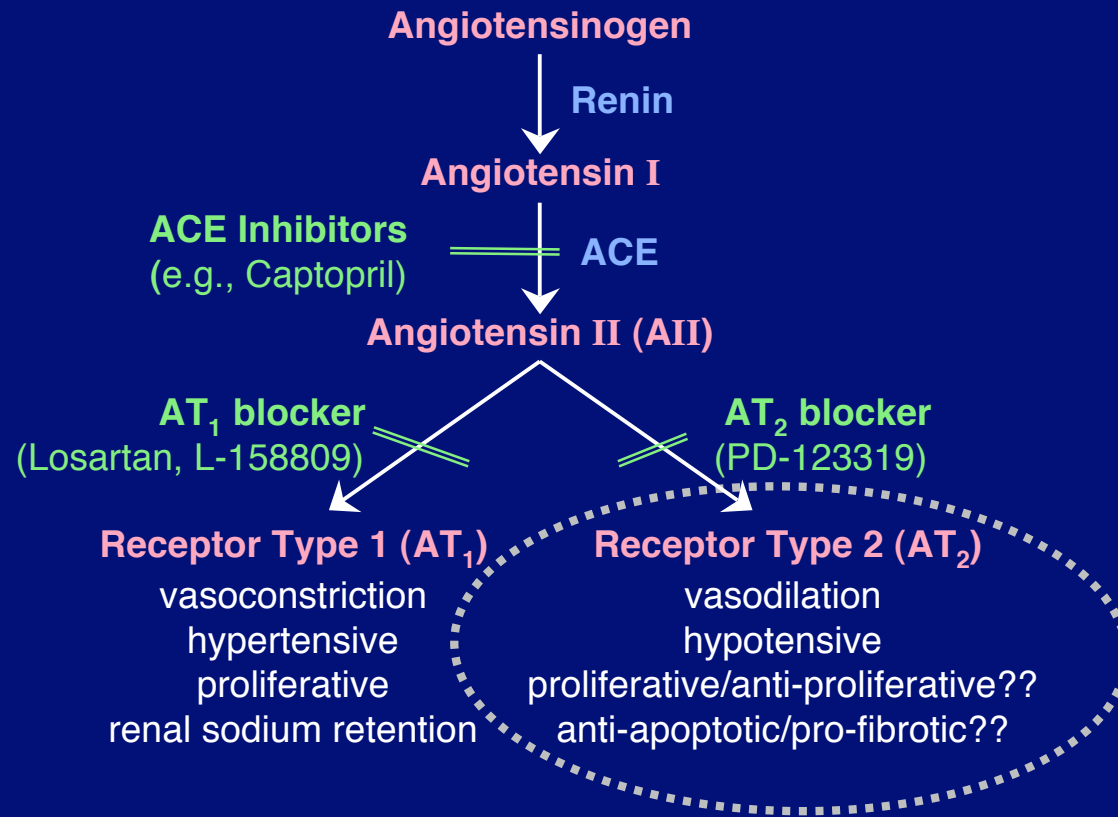


- AII blockers are more effective than ACE inhibitors in mitigating radiation nephropathy.

ACE inhibitor (Captopril) DMF: 1.15 (1.10 - 1.25)

AT₁ blocker (L-158809) DMF: 1.30 (1.20 - 1.35)

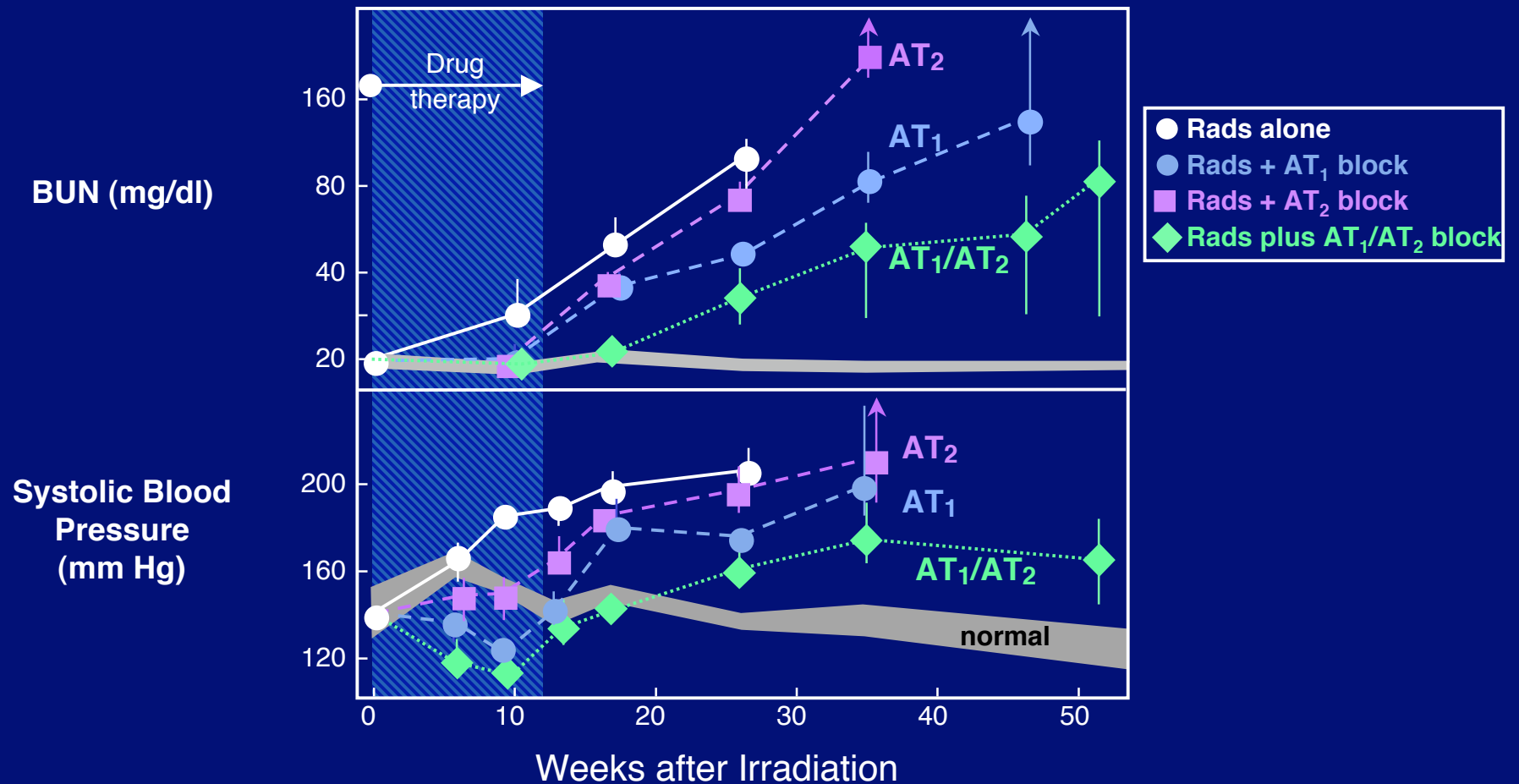
A Role for the AT₂ Receptor?



- **Blocking AT₁ causes unopposed over-stimulation of the AT₂ receptor.**
 - Maybe the AT₁ blocker is working via stimulation of the AT₂ receptor.
 - So blocking AT₂ could be bad.

Both AT₁ and AT₂ Blockers are Effective Mitigators

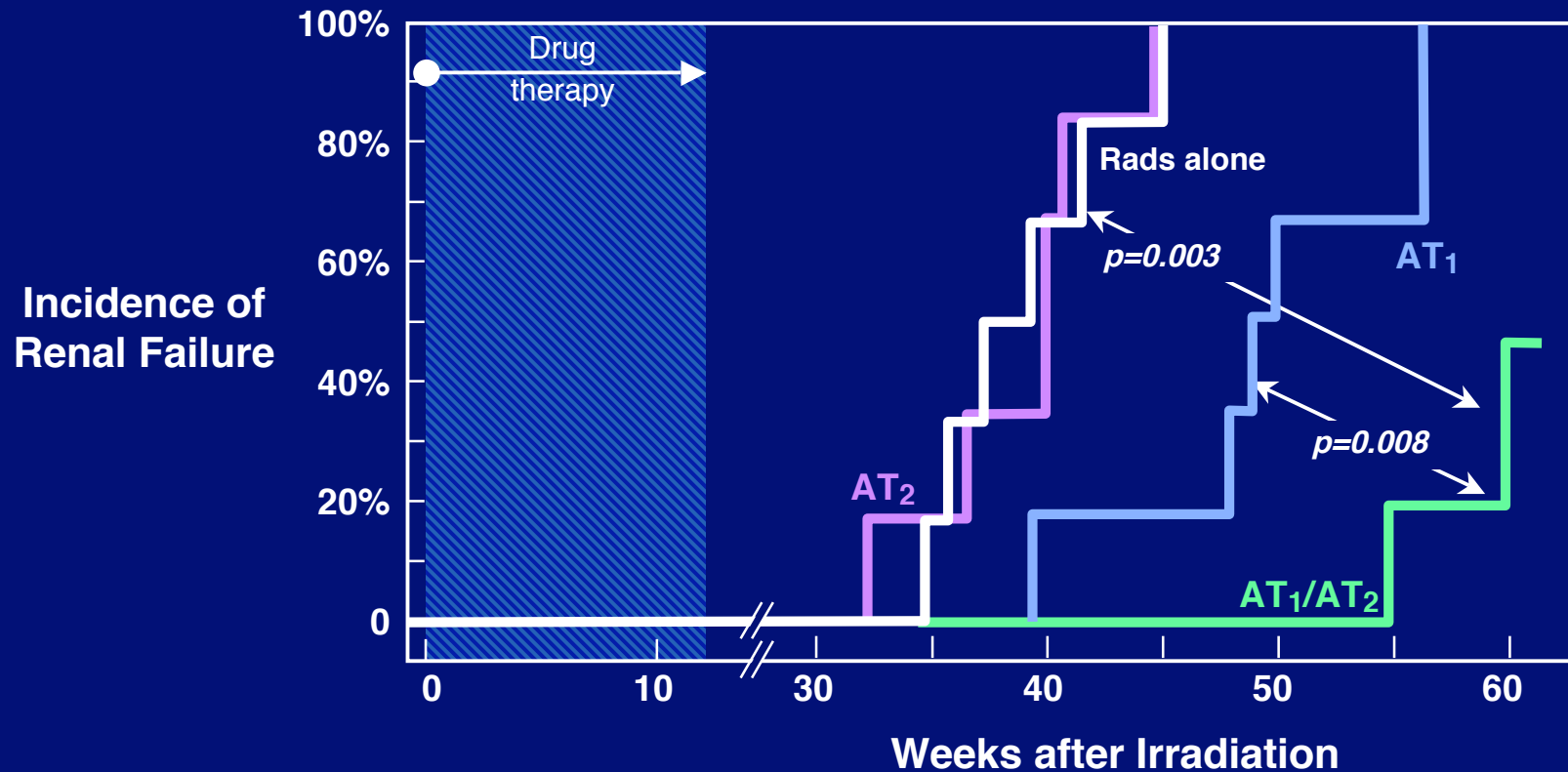
(Moulder et al., Rad Res 2004)



- The combination is more effective than either is alone.

AT₂ Blockade and Renal Failure

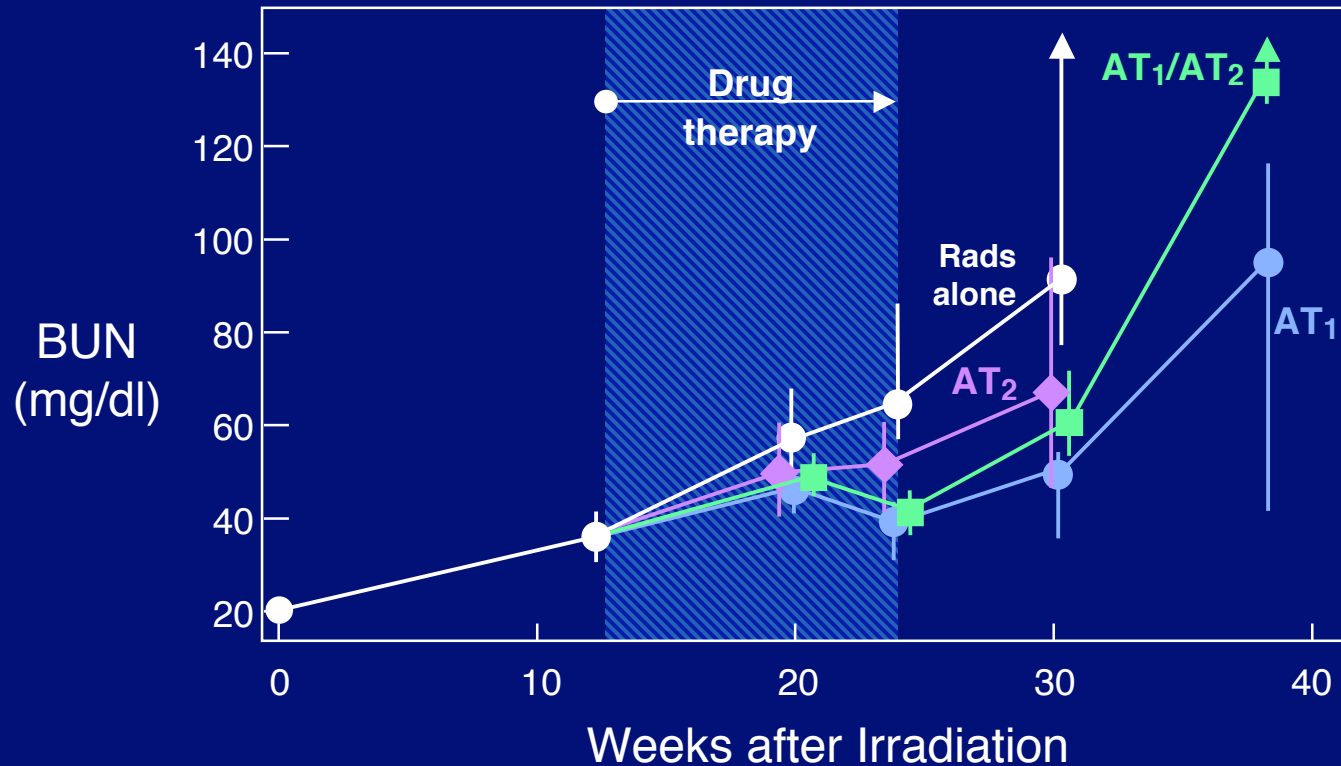
(Moulder et al., Rad Res, 2004)



- The acute attenuation of renal dysfunction seen for the AT₂ blocker alone **DOES NOT** translate into reduced late injury.

Do AT₂ Blockers Work in Treatment?

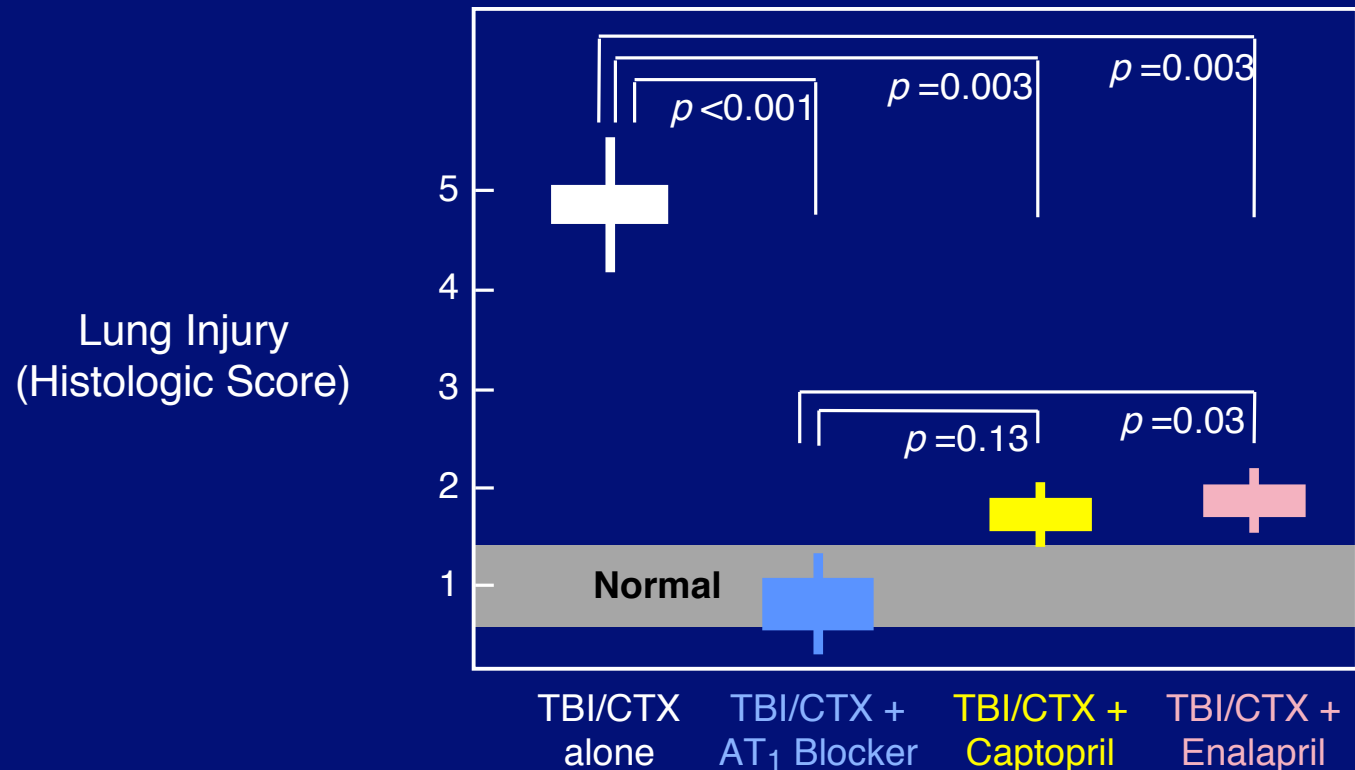
(Moulder et al., in press)



- **The AT₂ blocker may have some efficacy in treatment.**
 - The AT₂ blocker does not enhance the AT₁ blocker in treatment.

The RAS and Pneumonitis Induced by BMT Conditioning

(Molteni et al., Int J Rad Biol, 2000)

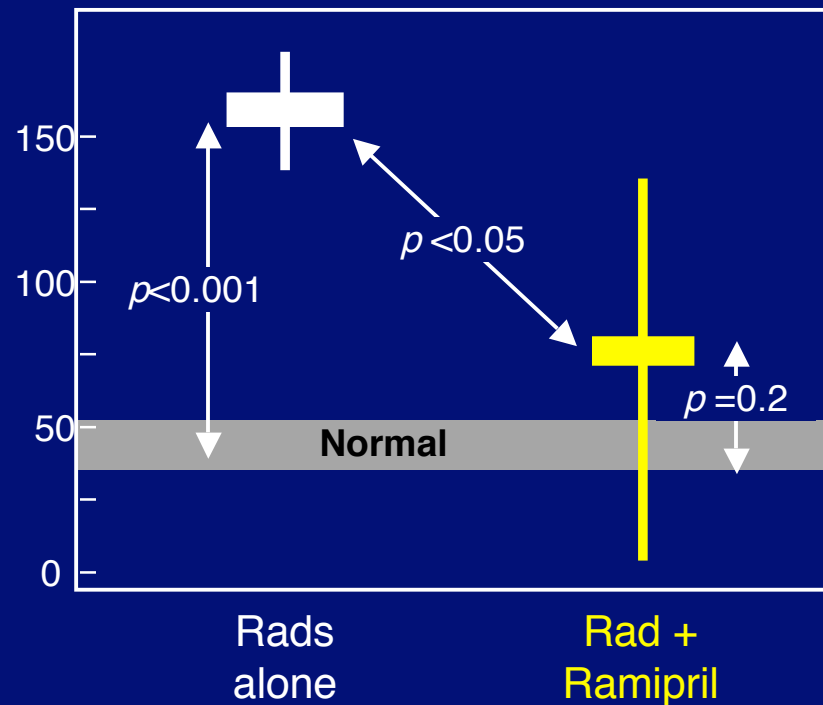


- **AT₁ blockade is more effective than ACE inhibition in mitigation of lung injury.**
 - Neither ACE inhibitors nor AT₁ blockers have been tested in treatment regimens.
 - AT₂ blockers have not been assessed in lung.

ACE Inhibitors in Mitigation of Optic Neuropathy

(Kim et al., Rad Res, 2004)

Delay of Visual Evoked Potential (msec) At 6 Months After Rads



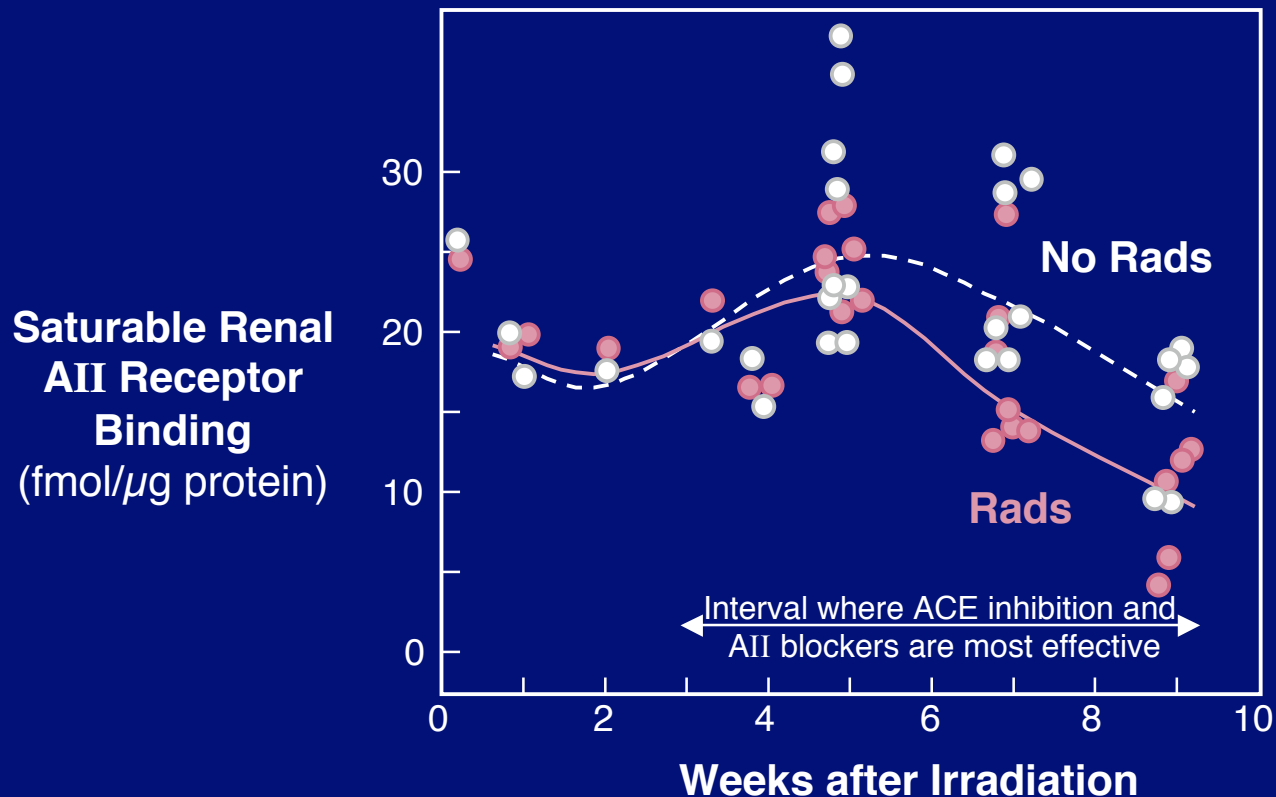
- **Ramipril (an ACE inhibitor) started 2 weeks after a single dose of 30 Gy and continued until 6 months.**
 - Treatment regimens not yet assessed
 - AII blockers not yet assessed

How Is RAS Antagonism Working?

- **It is not a strictly anti-hypertensive effect.**
 - Other classes of anti-hypertensive don't work in mitigation.
 - A high-salt diet works in mitigation despite exacerbating the radiation-induced hypertension.
- **Radiation does not cause up-regulation of the RAS in any obvious way.**
 - Renin, AII, ACE and aldosterone levels are unaffected by irradiation.
- **RAS antagonism probably does not work via suppression of radiation-induced proliferation.**
 - A high-salt diet works in mitigation without preventing radiation-induced tubular or glomerular proliferation.
- **What's left?**
 - Radiation induced up-regulation of AII receptors.
 - AcSDKP
 - Bradykinin
 - Angiotensin metabolites
 - Aldosterone

Does Radiation Up-Regulate AII Receptors?

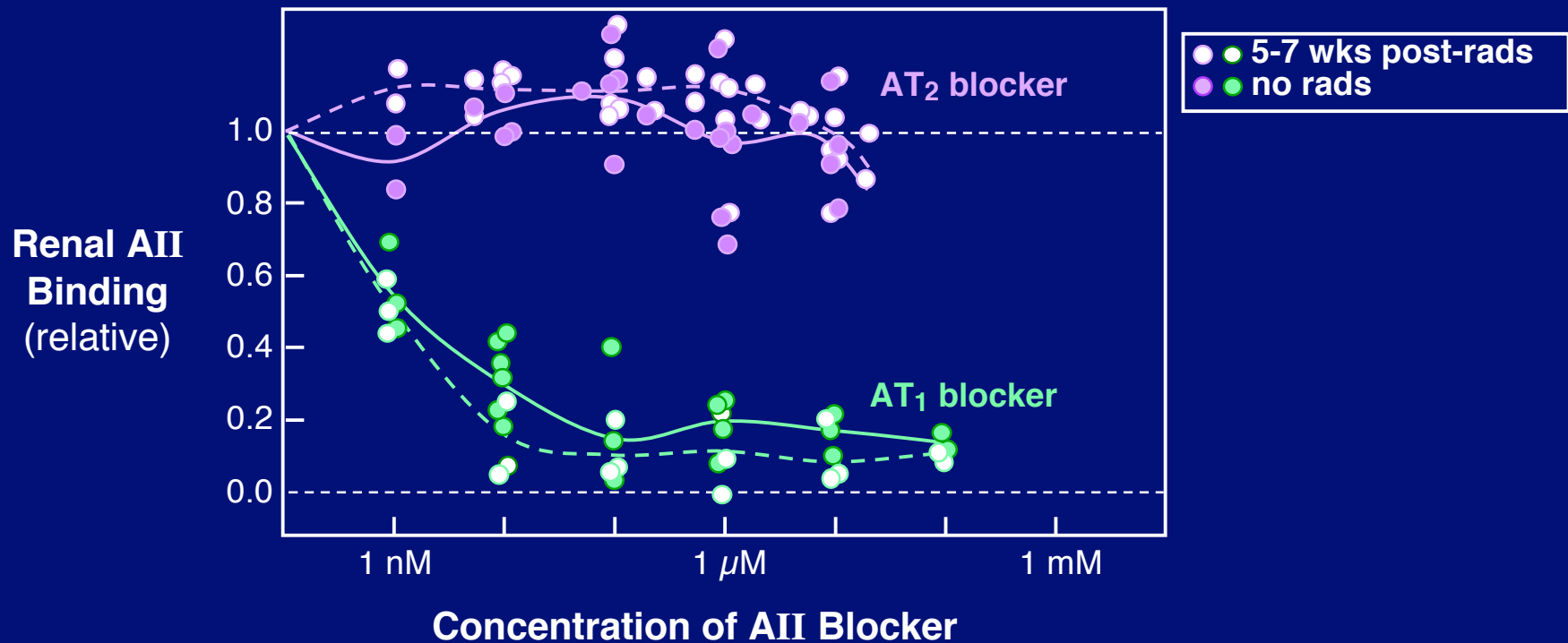
(Moulder et al., unpublished)



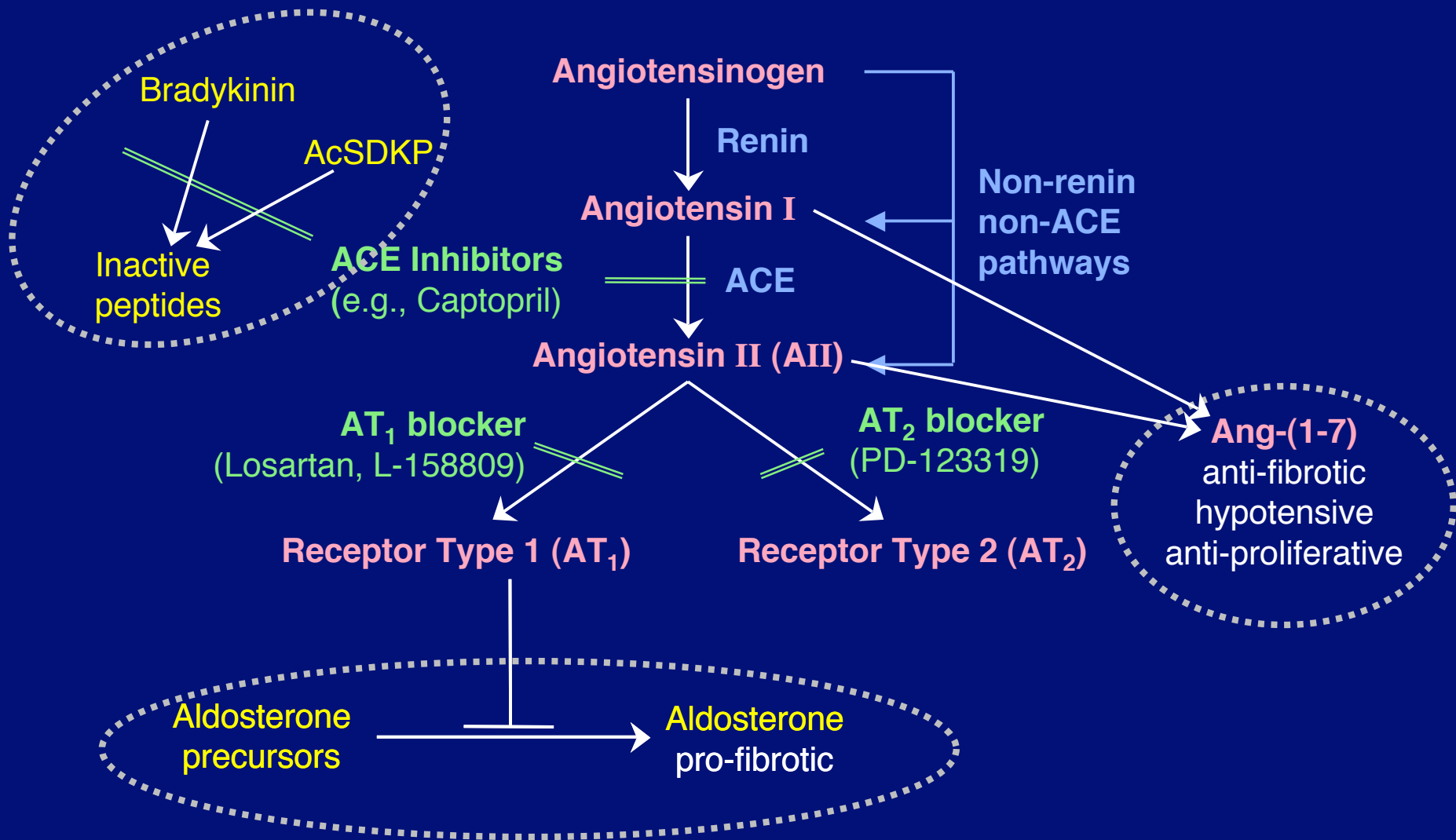
- **AII receptor binding was not significantly elevated over the interval (3-9 wks) when AII blockers are most effective in mitigation of radiation nephropathy.**
- **AII receptor density (B_{max}) is decreased after irradiation.**

AT₁ vs. AT₂ Receptors in Irradiated Kidney

(Moulder et al., unpublished)



- **Irradiation does not cause up-regulation of either AT₁ or AT₂ receptors.**
 - The AT₁ blocker blocks most AII receptor binding.
 - The AT₂ blocker shows no activity at μM levels.



AcSDKP

- **ACE inhibition leads to a rise in AcSDKP.**
 - AII receptor blockage may also cause a rise in AcSDKP, as AT₁ blockade causes a rise in AII which may in turn cause down-regulation of ACE.
- **AcSDKP may be a renal anti-fibrotic** (Peng et al., 2001, 2003; Rhaleb et al., 2001).
 - And it may inhibit renal cell proliferation (Azizi et al., 1996; Yoshioka et al., 1998; Iwamoto et al., 2000).
- **Could the effect of ACE inhibitors in radiation injury be mediated by AcSDKP** (Kim et al., 2004)?

	Urine AcSDKP at 4 wks	Urine AcSDKP/creatinine ratio [§] at 8 wks	BUN at 17 wks	Renal Failure at 35 wks
Rads alone	39±3 nM	2.6±0.6 nM/mM	51±13 mg/dl	4/10
Rads + Captopril*	94±40 nM	15.2±6.6 nM/mM	35±15 mg/dl	0/5
Rads + AcSDKP [‡]	117±4 nM	8.7±0.7 nM/mM	51±13 mg/dl	3/5

*50 mg/kg/day from 1 to 9 wks

[‡]0.8 mg/kg/day from 1 to 9 wks

[§]To correct for differences in water consumption

Bradykinin

- **As with AcSDKP, both ACE inhibitors and AT₁ blockers should lead to a rise in bradykinin levels.**
- **In other models of renal injury the drop in AII and the rise in bradykinin may act synergistically** (e.g., Siragy et al., '93; Zhu et al., '95; Tanaka et al., '94).
- **Could the effect of ACE inhibitors in radiation injury be mediated by kinins?**
- **We have not found a good way to test this hypothesis.**
 - The *in vivo* half-life of bradykinin is so short that chronic infusion may not be practical.
 - Bradykinin analogs are too expensive for chronic *in vivo* studies.
 - Bradykinin blockers are not widely available.
 - Mice are a very poor model for radiation nephropathy, so the transgenics don't help.
 - **Brown Norway Katholiek rats are deficient in kininogen.**
 - Not an easy experiment to design.
 - No commercial source
 - Abnormal renal function compared to wild-type Brown Norway rats

Aldosterone

- **Aldosterone promotes nephrosclerosis in several rat models.**
 - Aldosterone receptor antagonism (spironolactone) blunts activation of the RAS.
- **In a rat radiation nephropathy model, Brown et al. (2000) reported:**
 - Spironolactone reduced proteinuria and fibrosis.
 - Spironolactone enhanced the efficacy of an AT₁ blocker.
- **In our model:**
 - Aldosterone levels are not increased after irradiation.
 - Spironolactone had no effect on the development of radiation-induced proteinuria.
- **But:**
 - We have not yet proven that we gave spironolactone at a biologically effective dose.
 - We have not yet tried it in combination with either an ACE inhibitor or an AT₁ blocker.

AII Metabolites

- **Ang-(1-7) is an active metabolite of both AI and AII.**
 - It stimulates the proliferation of multipotential and differentiated progenitor cells in cultured bone marrow and human cord blood.
 - It may act as an endogenous inhibitor/antagonist of ACE and the AT₁ receptors.
 - It may be an anti-fibrotic.
- **AIII, AIV and des-Asp-AI regulate renal vascular resistance by activation of AT₁ receptors.**
- **Ang-(1-7), AIII, AIV levels may rise when the AT₁ receptor is blocked.**
 - The effect of ACE inhibition on these metabolites is unclear.

Mitigation and Treatment of Chronic Radiation Injury

- Mitigation and treatment of chronic radiation injuries is clearly possible.
- But the mechanism underlying the efficacy of RAS antagonism in mitigation and treatment of chronic radiation injuries is not clear.
 - It is not due to radiation-induced up-regulation of the RAS.
 - It is not due to elevation of AcSDKP levels (unpublished).
 - It is probably not mediated via aldosterone (in progress).
 - A bradykinin connection has not been ruled out.
 - A role for AII metabolites has not been ruled out.
- Efficacy of RAS suppression in treatment of radiation pneumonitis or neuropathy is unknown.
- Clinical efficacy of mitigation is unproven.

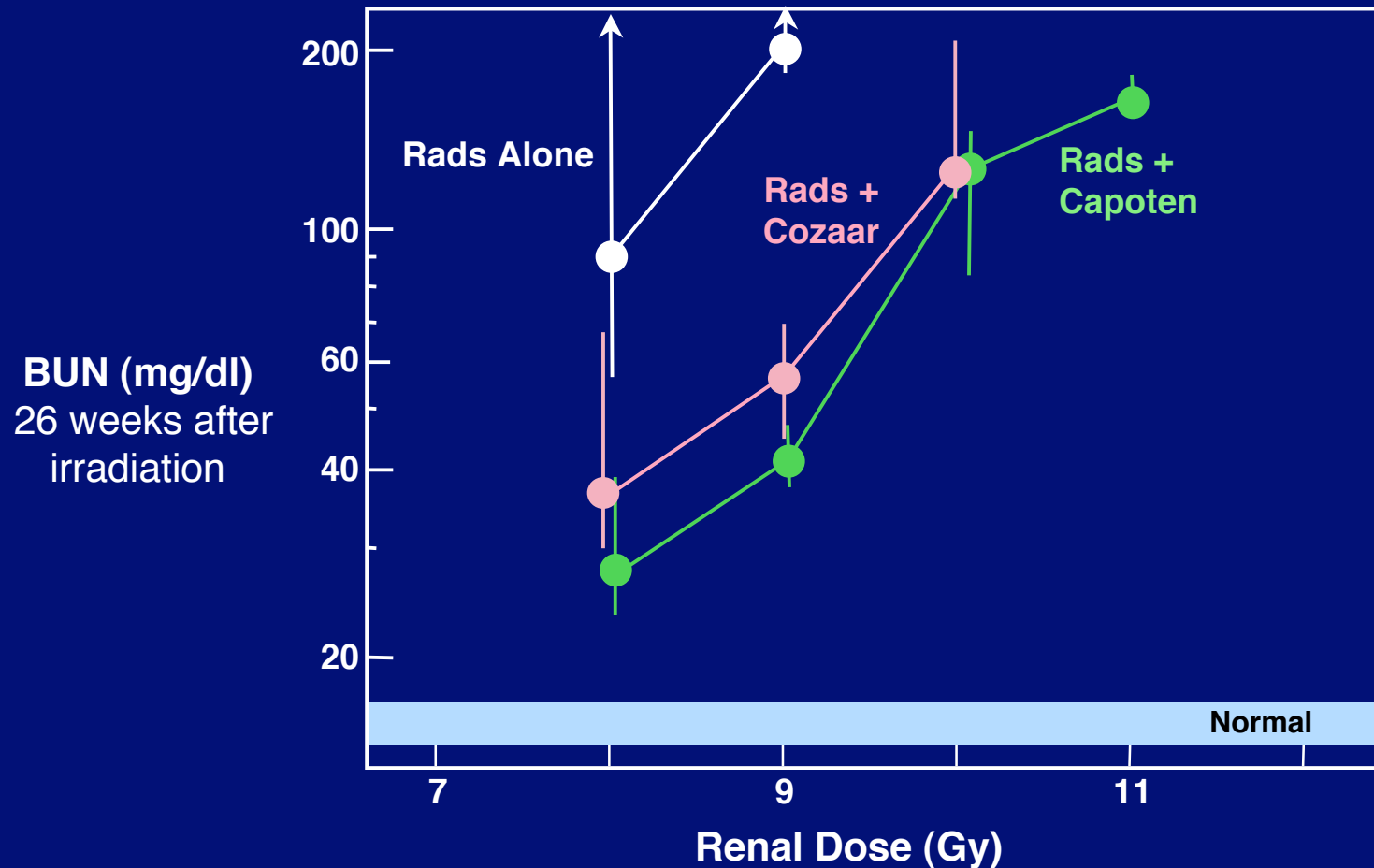
Issues Raised by the Radiological Terrorism Countermeasures Program

- **The AII blocker we had been using is not approved for human use.**
 - It is an analog of Losartan.
- **We are using the ACE inhibitor at doses above those commonly used in humans as anti-hypertensive agents.**
- **We have shown efficacy only after fractionated irradiation.**
 - Most radiological terrorism scenarios will be single dose at high or low dose rates.
- **We only have data for one species.**
 - FDA requires 2 species (one of which cannot be a rodent).
- **We have not shown which approach (ACE inhibition vs. AII blockade) is “best”.**
 - We have to make a choice before moving to a large animal (dog) model.

New Mitigation and Treatment Design

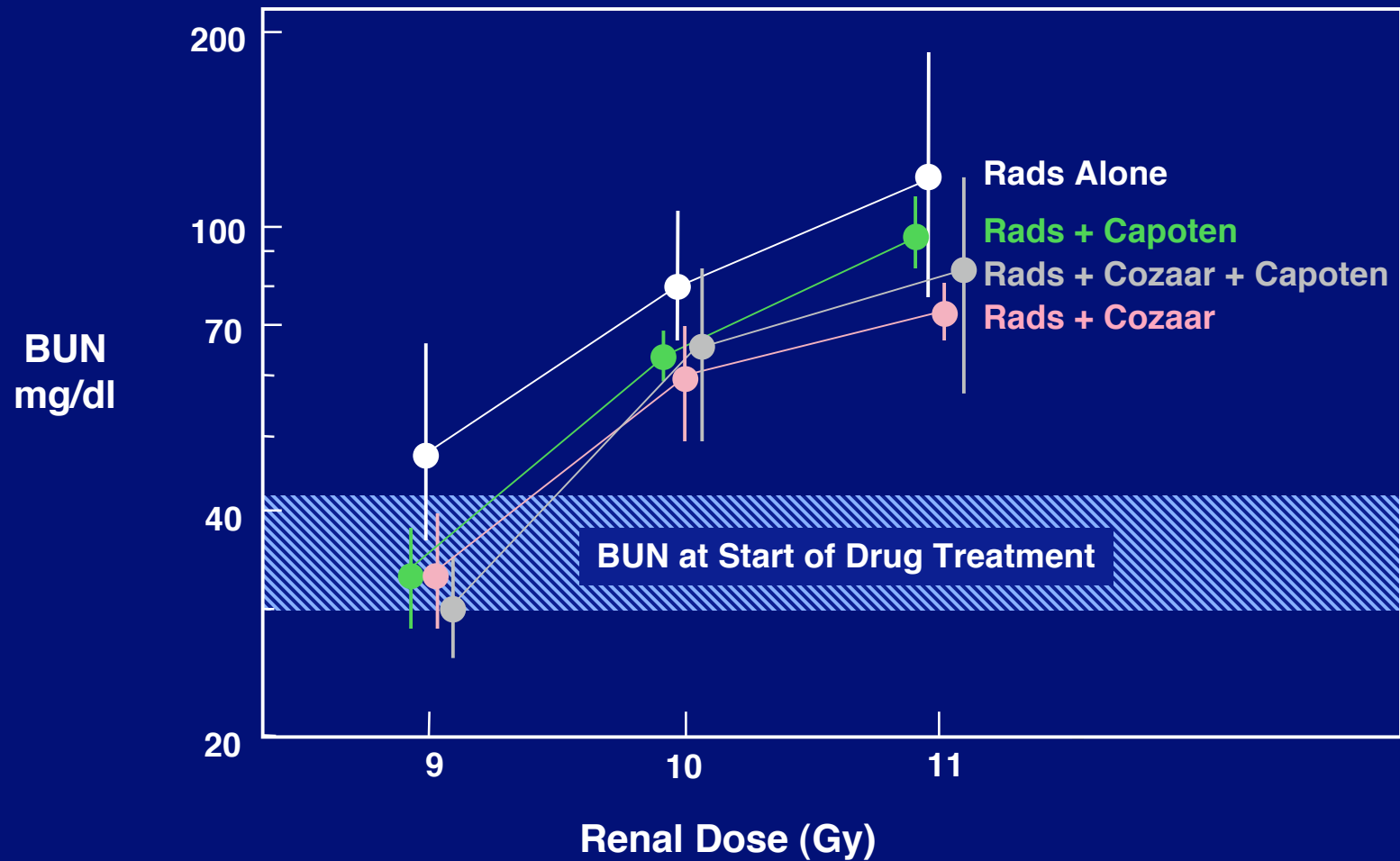
- **Using Captopril (Capoten®) and Losartan (Cozaar®).**
- **Drug doses matched to human use on a g/m²/day basis.**
 - **Losartan**
 - Human: 50-100 mg/day equals 30-60 mg/m²/day
 - Rat: 100 mg/l in drinking water equals 40-60 mg/m²/day
 - **Captopril**
 - Human: 75-150 mg/day equals 45-90 mg/m²/day
 - Rat: 150 mg/l in drinking water equals 60-90 mg/m²/day
- **Single dose of radiation.**
- **Bone marrow transplant within 1 day.**
- **Mitigation starts at 10 days and continues until 6 months.**
- **Treatment starts when BUN exceeds 25 mg/dl and continues indefinitely.**

Mitigation: Capoten[®] vs. Cozaar[®]



- Captopril and Losartan are effective in mitigating radiation nephropathy.
ACE inhibitor (Capoten) DMF: 1.18 (1.10 - 1.28)
AII blocker (Cozaar) DMF: 1.15 (1.07 - 1.21)

Two-Month Treatment: Capoten[®] vs. Cozaar[®]



What Next on Mitigation?

- **Continue follow-up to see how long we are delaying renal failure.**
 - Or whether we might even be preventing it.
- **Test the best of these two against a catalase/SOD mimetic.**
 - If Merck (which owns Cozaar®) is not interested in pursuing a label addition, we will have to go with captopril, which is off-patent.
- **Test the best of the three agents:**
 - In the rat model after low dose-rate irradiation.
 - In a dog model after high dose-rate irradiation.

What Next on Treatment?

- **Evaluate additional radiation doses.**
 - To see if there is a dose so high that treatment will not work.
- **Continue to follow-up to see how long we are delaying renal failure.**
 - Or whether we might even be preventing it.
- **Test the best of these two against a catalase/SOD mimetic.**
 - Again, if Merck (which owns Cozaar®) is not interested in pursuing a label addition, we will have to go with captopril, which is off-patent.
- **Test the best of the three agents in a dog model.**
 - We cannot assume that the best agent for mitigation will be the best agent for treatment.
 - In fact, we have data strongly indicating that the mechanisms of mitigation and treatment are NOT the same.

Summary

- **We have established efficacy for both mitigation and treatment of radiation nephropathy.**
- **With:**
 - an FDA-approved AII blocker at a clinically-applicable dose.
 - an FDA-approved ACE inhibitor at a clinically-applicable dose.
 - radiation schedules and doses that are relevant to both radiation oncology and radiological terrorism.
 - an established small animal model.
- **What we have not done:**
 - Established relative efficacy of the agents for treatment.
 - Established efficacy in a large animal model (an FDA requirement).
 - Established efficacy under GMP and GLP conditions (an FDA requirement).
 - Secured commitment of a relevant pharmaceutical company.
 - Figured out the mechanism.