Spring 2014 undergraduate students note:

- **This case was prepared by three undergraduate students in Chemistry and BME. It scored 49 out of 50 pts possible for the three students.**
- **The paper is longer than it should be, but is thorough.**
- **Individual student contribution (and score) was indicated by the students, but has been redacted here.**
- **Please be sure to include notation of contribution and sections responsible for on your written cases.**

Sildenafil

Background

Sildenafil is a phosphodiesterase inhibitor that acts as a vasodilator through its interaction with the nitric oxide signalling cascade. [N1] It was approved by the FDA for the treatment of erectile dysfunction in 1998. [N3] Sildenafil was approved for a second indication, pulmonary arterial hypertension, in 2005 and is branded under the name Revatio.[N1] It is a relatively widely prescribed drug, grossing over $1 billion in 2010. [N2] Though the patent was originally to expire in March of 2012, Pfizer, the parent company, was recently able to extend their patent to 2019 by relying on a method-of-use patent. [N4]

Discovery and Development

The study of atrial natriuretic peptide (ANP), a potent endogenous vasodilator, in addition to mechanistic knowledge of nitric oxide (NO) signalling helped lead to the development of sildenafil.[N5] ANP is a peptide hormone involved in the regulation of a variety of functions including renal and cardiac function while NO is a small diffusible molecule important for local vasodilatory signalling. Both ANP and NO were known to increase cyclic GMP (cGMP) levels , and thus inhibiting the breakdown of cGMP presented itself as an attractive target for the creation of new vasodilatory drugs. [N5] The enzymes responsible for the breakdown of cGMP are phosphodiesterases (PDEs). [N5]

Initially Pfizer was hoping to treat hypertension or angina (poor blood flow to cardiac muscle) with a PDE inhibitor. At the time, the drugs available for the treatment of angina were nitrates, which activate the NO signalling pathway (see Figure 1) by directly adding NO. However, prolonged use of nitrates leads to tolerance.[N1] This too made PDE inhibition an attractive goal. They chose to target PDE5 specifically because it was known to selectively catalyze the conversion of cGMP to GMP where other PDEs also catalyze cAMP breakdown. [N1]

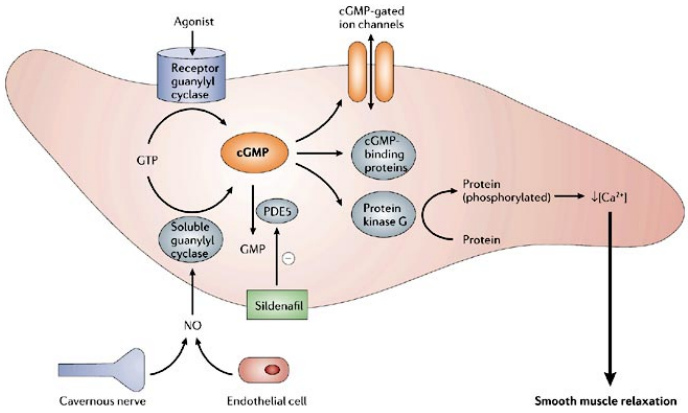


Figure 1. *"The figure shows stimuli promoting the synthesis of cGMP, downstream intracellular signalling targets modulated by cGMP and the role of phosphodiesterases (PDEs) in cGMP breakdown. This pathway mediates relaxation of vascular smooth muscle and penile erection (only upon sexual stimulation) and pulmonary vasodilatation (continuously). Smooth muscle relaxation is in part mediated via protein kinase G (PKG) activation, subsequent potassium channel opening and reductions in intracellular calcium levels. PDE5 is the target for sildenafil and other PDE5 inhibitors in the treatment of chronic vascular disorders. cGMP, cyclic guanosine monophosphate; GMP, guanosine monophosphate; GTP, guanosine triphosphate; NO, nitric oxide."* *[N1]*

Comment [DS1]: Why in quotes, because directly from the paper??

During sildenafil's clinical trials as a treatment for angina, doubt was cast upon its effectiveness and marketability. It showed a relatively short half (4 hours, meaning multiple administrations per day) and would have to be contraindicated for patients on nitrates. [N3] However, one of the commonly noted "adverse effects" during these trials was penile erection. In the early 1990s when sildenafil was being developed, the only treatments available for erectile dysfunction were rather invasive: penile implants, vacuum pumps, and injections directly into the corpus cavernosum (the spongy tissue that becomes engorged with blood during erection). [N3] The possibility of breaking into a relatively untapped market led Pfizer to shift sildenafil's focus from angina and hypertension to erectile dysfunction. [N1]

Production of an erection had been shown to proceed through the vasodilatory mechanisms outlined in figure 1 (note that in the corpus cavernosum it is neuronal production of NO that stimulates the vasodilation, not endothelial production). [N1] This knowledge, in addition to finding that the corpus cavernosum is enriched with PDE5 (the target of sildenafil) allowed Pfizer to put sildenafil into clinical trials for ED in 1993. [N1]

Molecular Targets and Mechanism

The primary action of sildenafil is to inhibit PDE5, but it inhibits other phosphodiesterases as well, albeit less potently. Table 1 summarizes the locations of the various PDE forms and the concentration of sildenafil needed to inhibit them.

PDE	Subfamily (number of variants)	Tissue	Role	Sildenafil IC_{50} (μM) (fold selectivity)
1	a (4)	Vascular smooth muscle, testis, heart, olfactory, epithelial smooth muscle	Proliferation, modulation of olfaction	0.28 (82)
	b (1)			
	c (5)			
2	a (3)	Broad, brain, adrenal cortex	Ca^{2+} channel regulation, olfaction, platelet aggregation, aldosterone secretion	> 30 (> 8823)
3	a (1)	Broad, adipose, liver, cardiac muscle, vascular smooth muscle, platelets	Cardiac contractility, insulin secretion, lipolysis	16.20 (4765)
	b (1)			
4	a (8)	Broad, neural, endocrine, monocytes, macrophages, T - lymphocytes, neutrophils, airway and vascular smooth muscle	Imune and inflammatory modulation, Smooth muscle contractility, depression	7.68 (2259)
	b (3)			
	c (4)			
	d (5)			
5	a (3)	Vascular smooth muscle, penis, lung	Penile erection, smooth muscle contractility	0.0034 (1)
6	a (1)	Rod and cone photoreceptor cells	Vision	0.04 (12)
	b (1)			
	c (1)			
7	a (3)	T -lymphocytes, monocytes, eosinophils, airway and vascular smooth muscle, lung fibroblasts, cardiac and skeletal muscle	T-lymphocyte activation, proliferation, cardiac and skeletal muscle metabolism	21.30 (6264)
	b (3)			
8	a (1)	Broad, testis, thyroid gland, brain	T-cell activation	29.80 (2578)
	b (5)			
9	a (1)	Broad, spleen, intestine, kidney, heart, brain	Maintain cGMP levels, natriuresis, vascular tone	2.61 (768)
10	a (2)	Broad	Unknown	9.80 (2882)
11	a (4)	Broad, testis, sperm, prostate, kidney, skeletal muscle, vascular smooth muscle	Sperm capacitation, sperm motility, acrosome reaction	2.73 (803)

Table 1. Left-most columns specify type of PDE and isoforms. Middle columns show tissue distribution and putative function. Final column shows IC50 values and in parentheses the relative inhibition compared to PDE5. [N3]

Comment [DS2]: Table legends at the top of tables

At concentrations used to inhibit PDE5, the most relevant off-target interaction is sildenafil's inhibition of PDE6. Sildenafil inhibits PDE6 at a concentration approximately one order of magnitude higher than it inhibits PDE5. PDE6 is found exclusively in photoreceptor cells, and is involved in signal transduction. This interaction is the putative cause for some of the vision-related side effects associated with sildenafil. (see Adverse Reactions)

A study published in the Journal of Medicinal Chemistry analyzed the binding pockets of 19 different PDE forms and highlights the difficulty in creating a truly selective PDE inhibitor. (See Figures 2 and 3)

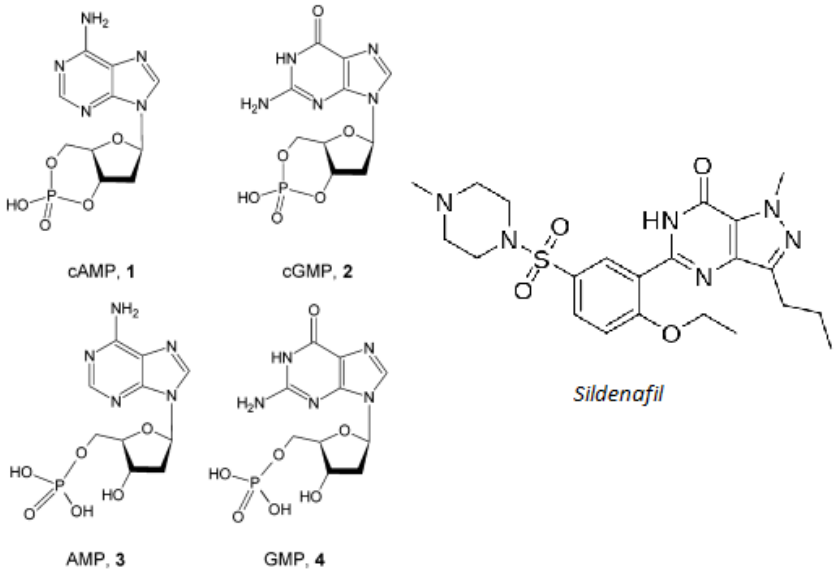


Figure 2. Comparison of the substrates and products of PDEs with sildenafil. Only the conversion of cGMP to GMP is catalyzed by PDE5, against which sildenafil is most potent. [N6], wikipedia.

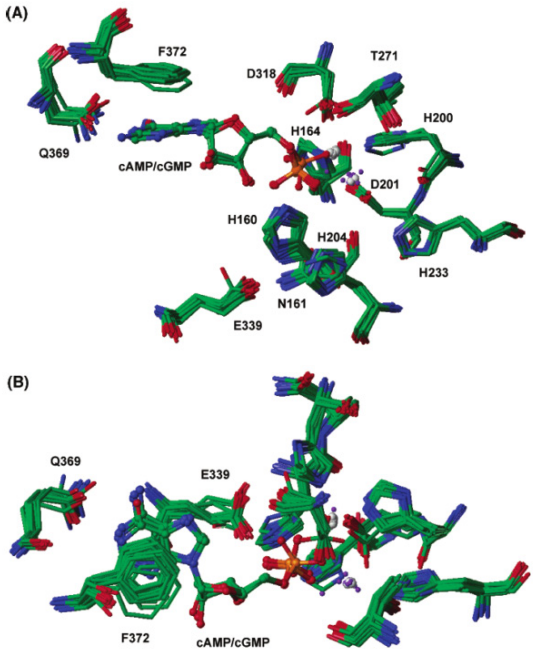
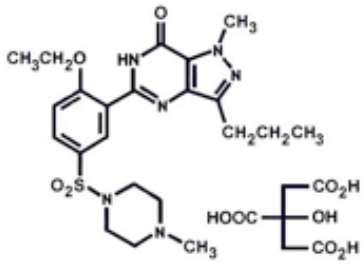


Figure 3. "Superimposition of 19 PDE enzymes demonstrating the close fit of the 11 invariant residues in the active site. This view is from the perspective of a ligand approaching the protein.

cAMP and cGMP have been left in place to assist orientation. (B) Alternative view of the superimposition of the 19 PDE enzymes looking down over the phenylalanine at the roof of the binding site. Ligands would approach the binding site from the bottom of the diagram." [N6]

Despite the extraordinarily similar binding pocket structures, sildenafil is a relatively selective agonist of PDE5. This selectivity helps make it an effective drug and limit its side effect profile.



1-[4-ethoxy-3-(6,7-dihydro-1-methyl-7-oxo-3-propyl-1 H-pyrazolo [4,3-d] pyrimidin-5-yl) phenylsulphonyl]-4-methylpiperazine citrate

Chemistry

The chemical properties of Sildenafil give the drug its fast mode of action and a bioavailability of 41%. We can see there are 10 reactive bases in the molecular structure, which violates Lipinski's rule to a small extent. However, as a whole the molecule is very non-polar and only contains a very acidic pyrimidine ring. It is distributed as a citrate salt to increase the solubility in water to 0.11mg/L. What gives the molecule its fast absorption of 92% is that the pKa values of the sulfonamide group and imidazole ring are very close to physiological pH. This causes the molecule to partition between ionized and nonionzed forms in a 1:1 ratio allowing for maximum aqueous solubility and penetration through the cell membranes. [Z5]

Comment [DS3]: Why does this indicate 'fast'?

[Z6]

Pharmacokinetics

	Sildenafil Dose			
	25 mg (n = 224)	*50 mg (n = 450)*	*100 mg (n = 591)*	*200 mg (n = 115)*
AUC (ng·h ml^{-1})	464 ± 175	950 ± 346	1963 ± 860	5486 ± 1965
C_{max} (ng ml^{-1})	84.4 ± 80.5	157 ± 49.2	328 ± 237	903 ± 287
T_{max} (h)	1.09 ± 0.89	1.03 ± 0.76	1.16 ± 0.99	0.99 ± 0.82
$t_{1/2}$ (h)	3.60 ± 0.72	3.67 ± 0.69	3.82 ± 0.84	3.70 ± 0.73
CL/F (l h^{-1})	58.2 ± 8.31	57.4 ± 8.29	56.5 ± 8.60	56.9 ± 8.51
V/F (l)	295 ± 29.7	297 ± 29.4	303 ± 36.4	296 ± 29.6
F_{rel}	1.04 ± 0.27	1.05 ± 0.25	1.05 ± 0.29	1.50 ± 0.36
k_a (h^{-1})	12.9 ± 26.1	14.9 ± 27.8	14.1 ± 25.2	20.8 ± 27.4
k_e (h^{-1})	0.200 ± 0.038	0.195 ± 0.035	0.188 ± 0.038	0.194 ± 0.035

AUC = area under the curve; CL/F = apparent clearance; V/F = volume of distribution; k_a = first-order absorption constant; C_{max} = maximum observed concentration; T_{max} = time to C_{max}; $t_{1/2}$ = half-life; F_{rel} = relative bioavailability.

Table 2. Pharmacokinetic parameters as a function of sildenafil dose.[Z1]

A meta study was conducted using five phase III trials that had gathered pharmacokinetic data. Patients were asked to not eat, drink, exercise, or take any other drugs 36 hours prior to testing to obtain the most accurate result. Viagra seemed to demonstrate first order kinetics with the AUC and C_{max} increasing proportionally with dose. Strong evidence of this was demonstrated when each trial revealed similar values across all measurements.

Comment [DS4]: Be consistent in use of drug name

However, the absorption constant had around a 200% variability, which is likely caused by sampling and dosage history inaccuracies why??.[Z1] The clearance of the drug was affected by a few factors including age, CYP3A4 inhibitors, and liver function. The presence of food only affected t_{max} significantly by increasing it to 2 hours. Patients are recommended to take the pill on an empty stomach to achieve the fastest onset of effects. If individuals were taking CYP3A4 enzyme inhibitors this would inhibit metabolism of sildenafil and hence a reduced clearance. If patients were older they had weakened liver function and therefore higher levels of AST(aspartate transaminase) This is a metabolizing enzyme in the liver and increased levels where, in plasma? indicate liver disease and would decrease clearance. For each 10 unit increase in AST a 6% decrease in clearance was observed and for every 10 year increase in age a 4% decrease was observed. [Z2]

Metabolism

Sildenafil is metabolized quickly in the liver predominantly by the CYP3A4 enzyme and much less so by CYP2C9. It has a short half-life where? of 4 hours and yields two main metabolites with UK-103,320 being half as potent as the drug and UK-150,564 being inactive (see figure 4 for structures). None of sildenafil is excreted unmetabolized in the urine or feces, as it is greatly reabsorbed to the blood stream from the the kidney and GI tract. The principle metabolism methods were N-demethylation to UK-103,320, apliphatic dehydroxylation to UK-150,564, and oxidation to minor metabolites.[Z3]

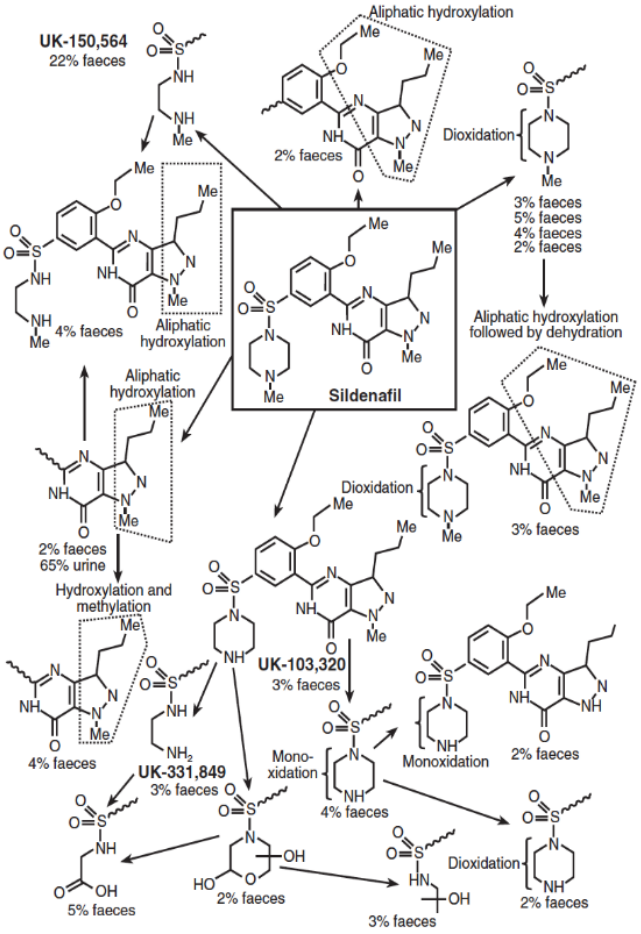


Figure 4. Overview of the metabolism of sildenafil. [Z3]

Adverse Reactions

ADVERSE EVENTS REPORTED BY ≥2% OF PATIENTS TREATED WITH VIAGRA AND MORE FREQUENT ON DRUG THAN PLACEBO IN PRN FLEXIBLE-DOSE PHASE II/III STUDIES

Adverse Event	Percentage of Patients Reporting Event	
	VIAGRA	PLACEBO
	N=734	N=725
Headache	16%	4%
Flushing	10%	1%
Dyspepsia	7%	2%
Nasal Congestion	4%	2%
Urinary Tract Infection	3%	2%
Abnormal Vision†	3%	0%
Diarrhea	3%	1%
Dizziness	2%	1%
Rash	2%	1%

†Abnormal Vision: Mild and transient, predominantly color tinge to vision, but also increased sensitivity to light or blurred vision. In these studies, only one patient discontinued due to abnormal vision.

Table 3. Occurrence of side effects during clinical trials. Dyspepsia refers to irritated stomach. [Z7]

Less than 2% of patients experienced serious side effects and most were mild such as headache and stomach pain. The sensitivity to light does have a molecular basis and is the only serious risk associated with sildenafil use (see figure 5). The increased cyclic GMP in these neurons causes increased glutamate release, which inhibits neighboring retinal function. This decrease in signaling from the eye to the brain causes the sensitivity to light and, in some cases a loss of differentiation between green and blue light. This blue tint in vision was observed in people taking large doses or who had decreased clearance of the drug and it goes away with discontinued use. [Z8]

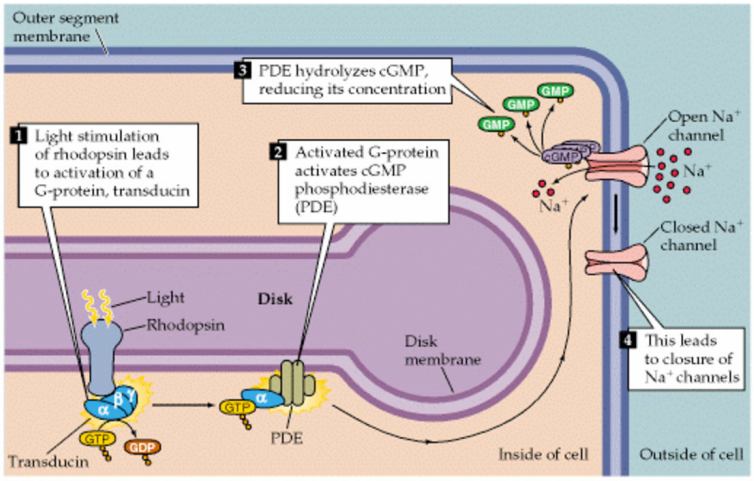


Figure 5. This figure shows rhodopsin inducing transductin entry into the retinal cell where it activates the PDE6 enzyme to hydrolyze cGMP. This hydrolysis causes sodium and calcium(not shown in figure) channels to close and reduce signaling [Z8]

Viagra may have an alternate use in treating priapism (an erection lasting more than 4 hours) in Sickle Cell Anemia patients. This is due to the sickled red blood cells destroying nitrous oxide producing met-hemoglobin cells. This decrease in nitrous oxide inhibits blood flow out of the penis once and erection is acquired. If not treated promptly damage to crucial arteries in the penis can be destroyed, which could make erections very difficult in the future. Sildenafil is being used to treat this problem by relieving the tension in the soft muscle tissue allowing blood flow back out of the penis.[Z4]

Clinical Trials

Comment [DS5]: This info should come before AEs

There were many different clinical studies on sildenafil, and there was a distinct difference between studies before patent and studies after patent. The clinical trials before patent were focused on safety and basic efficacy in normal populations (can an erection be achieved) while many post marketing studies looked at efficacy in special populations. Phase I-III trials were designed to look at the details regarding how sildenafil can treat ED and asked participants questions about penile erection rigidity or about patients' sexual performance. As for Phase IV trials, many present a different experimental aim where studies offer results that diversify sildenafil usage to different ED patients by proving its efficacy in patients with previous health problems, with different severities of ED, and also testing specifically to different demographics. Nonetheless, both studies before and after patent have the same essential characteristic of demonstrating that sildenafil is efficacious at treating ED.

One of the first clinical trials Pfizer constructed for sildenafil treating ED was a double-blind study in Sandwich, UK during 1996 [M1]. 12 patients were randomly selected, ages 36-63 each with ED for unreported reasons, to participate in two distinguished trials: one aimed for penile plethysmography analysis, and the other regarding daily erectile activity. Part one for penile plethysmography, or blood flow to the penis, involved a four-way cross analysis between the 12 patients with sets of three either receiving: placebo, 10 mg, 25 mg, or 50 mg. Patients were given their dosage at a supervised clinic, ask to wait 30 minutes, then given sexually suggestive material such as magazines and videotapes, and finally timed for how many minutes adequate blood flow was moving through the penis. The data was indirectly accumulated by judging how long the erection was rigid in both the base and tip of the penis, with rigidity implying that sildenafil is actively vaso-dilating the penis for blood flow to produce this rigidity. The time the penis was considered rigid was based on the criteria that the erection was rigid enough for sexual intercourse based on the International Index of Erectile Dysfunction (IIED). The results confirmed an increase in erection duration directly related to an increase sildenafil dosage with ED patients taking the placebo producing a mean erection for 1.6 minutes and patients taking 50 mg of sildenafil producing a mean erection for 11.2 minutes [M1].

Part two of the same study was tested at the patient's homes, where they were asked to keep "erection diaries" and to record the number of erections they would have after taking either a placebo or 25mg of sildenafil between the hours of 8:00PM and 11:00PM [M1]. They did this for seven straight days and recorded the mean number of erections for each day. The results demonstrated a significant increase in the number of erections for patients who took sildenafil compared to the placebo group, noticeably by peaks in the 0-2 hour post-dosing category (Fig. 5). Additionally, the study reported that peaks that were noticeably prevalent 8-10 hours after dosing "correspond to early morning erections". Based on the results the report concluded that sildenafil, "…is a well-tolerated and effective oral therapy for male erectile dysfunction…" in respect to both erection duration and frequency [M1].

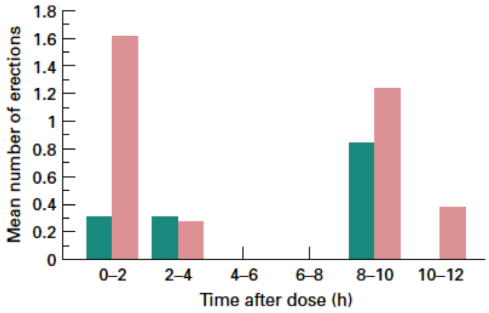


Figure 5. *Graph depicting Part 2 of the Phase I clinical trial tested in Sandwich, UK. Study was designed with two-way crossover between 12 erectile dysfunction patients. Patients that received the placebo are represented by the green, and patients that received 25 mg of sildenafil are represented by the pink [M1].*

Another pre-patent clinical trial, this one more like a Phase III trial by patient count, demonstrated a similar investigative technique to appeal to the ambiguous ED audience. The Massachusetts Medical Society did a 12-week and 24-week study for ED patients willing to take sildenafil prior to sexual activity with the requirement that they had a sexual partner for longer than six months [M2]. In the 12-week dose-response study, where 532 men were treated with oral sildenafil (25, 50, or 100mg) or placebo, Pfizer starting to use a questionnaire based on the Interaction Index of Erectile Function. This index consisted of various questions pertaining to erection ability during sexual intercourse graded on a scale 1-5 where 5 demonstrated easier ability to initiate an erection, maintain an erection, etc. and 1 being the opposite. They then summed these scores to create a “mean EF domain score”. Using this scoring system in the 12-week study they looked at placebo and sildenafil dosing regarding different questionnaire categories: erectile function, orgasmic function, sexual desire, intercourse satisfaction, and overall satisfaction. All but sexual desire (which showed no change in mean domain score compared to baseline between sildenafil and placebo dosing) showed a significant increase in “mean domain score” when patients with ED took sildenafil [M2].

The Massachusetts Medical Society also reported another investigation using a 24-week dose-response study of 205 men receiving placebo, 97 men receiving 25 mg of sildenafil, 105 men receiving 50 mg of sildenafil, and 102 men receiving 100 mg of sildenafil [M2]. Patients kept a sexual function log where they recorded the number of erections throughout the month, and graded the erection rigidity based on the IIED, scoring erection rigidity between 1-4. The results clearly indicated that sildenafil increased the mean number of erections per month and additionally showed that increased amounts of dosing also improved penile rigidity based on the proportion of grade 4 compared to grade 3 erections being greater than the placebo and lower doses (Fig. 6). Overall, these studies, along with many others, show the ease with which Pfizer was able to demonstrate sildenafil’s efficacy in treating ED after halting its development as a treatment for angina [M2].

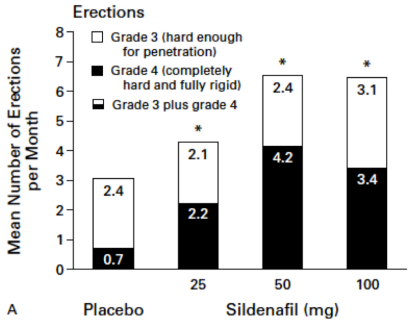


Figure. 6 *Graph depicting a Phase III clinical trial as a 24-week dose response study. 205 men received placebo, 97 men received 25 mg of sildenafil, 105 men received 50 mg of sildenafil, and 102 men received 100 mg of sildenafil. Number of erections were averaged based on the last 4 weeks of the study, and graded between 1-4 based on the International Index Erectile Dysfunction (IIED) [M2].*

Since 1998 many Phase IV trials have been organized with the aim to demonstrate that not only can sildenafil treat ED, but also sildenafil can treat a patient's ED regardless of the causative circumstances. One report that particularly stood out was a meta-analysis done in 2002 on 2667 patients with ED between the ages of 23 to 89 [M3]. Data was pooled from 11 double-blind, place-controlled, flexible-dose studies to assess sildenafil efficacy. They concluded that sildenafil improved erectile function compared to baseline, "…regardless of patient age, race, body mass index (BMI), ED etiology, ED severity, ED duration, or the presence of various comorbidities." They were able to demonstrate efficacy when patients had less severe comorbidities such as patients taking antidepressants and even when patients had more severe comorbidities such as blood pressure problems, heart disease, and vascular disease. (Fig. 7). Many other meta-analysis studies use similar dynamic approaches and produced similar results demonstrating sildenafil as an efficacious drug for treating ED [M3].

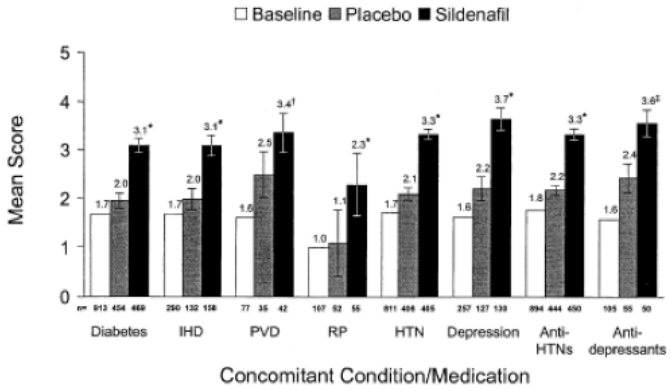


Figure 7. *Graph depicting results from a meta-analysis pooling data from 11 different different clinics across the United States. 2667 patients were questioned and scored based on polling from the International Index of Erectile Function; a higher mean score meant a more adequate erection for sexual interacourse. Patients were grouped based on prior medical conditions with the graph representing a group containing an average of both placebo and sildenafil baseline (white), placebo (grey), and sildenafil (black) [M3].*

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[Z7] http://www.pfizer.com/files/products/uspi_viagra.pdf

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