

Supplementary Material:<https://figshare.com/s/1477105d25e8809a0217>

GENE	ax2508 Mature Ventricular Cardiomyocytes (Transcript per kilobase million)	ax2518 Mature Atrial Cardiomyocytes (Transcript per kilobase million)
PDE10A	0.933249	0.554566
PDE11A	0.0324838	0.0418983
PDE1A	0.0172377	0.0458297
PDE1B	0.68122	1.09068
PDE1C	0.0756201	0.174333
PDE2A	0.0487482	0.136652
PDE3A	0.158159	1.00686
PDE3B	0.531229	0.64247
PDE4A	0.0755568	0.172701
PDE4B	0.371019	0.103569
PDE4C	0.0812053	0.410914
PDE4D	0.316264	0.288343
PDE5A	1.65885	2.18326
PDE6A	0.0903117	0.368927
PDE6B	4.79575	6.73855
PDE6C	0.060187	0.22712
PDE6D	3.05316	4.23302
PDE6G	0.15401	0.495772
PDE6H	0.0328429	0.038951
PDE7A	2.35514	2.62551
PDE7B	0.0323087	0.0711253
PDE8A	0.771781	1.27906
PDE8B	0.0828638	0.943161
PDE9A	0.157448	0.74975

Supplementary Table 1. RNA sequencing data from Axol's matured hiPSC derived cardiomyocytes used to create Table 3. RNA was extracted using Qiagen RNEasy Mini kits, strand specific libraries were prepared using Illumina mRNA library kits and then sequenced with 150bp paired-end read on NovaSeq6000. Sequenced data was then normalised by transcripts per millions to give the values shown. The creation and use of Axol's iPSC-derived cardiomyocytes was in accordance with all relevant ethical requirements.

Supplementary Discussion:

We have performed the following search on clinical trials.gov:

Condition/Disease: Atrial Fibrillation OR Atrial Fibrillation OR AF OR Atrial Fibrillation Persistent OR Atrial Fibrillation New Onset OR Atrial Fibrillation Paroxysmal OR Atrial Flutter OR Atrial Tachycardia | Intervention/Treatment: Phosphodiesterase Inhibitors OR phosphodiesterase type 5 inhibitor OR Phosphodiesterase 5 Inhibitor OR

Phosphodiesterase type 5 Inhibitor OR PDE5 OR PDE5 inhibitor OR PDE5i arm OR PDE5 Inhibitor OR PDE5- inhibitors group OR PDE5 inhibitor OR tadalafil OR PDE9i OR Vardenafil OR Levitra OR BAY38-9456 OR Vardenafil ODT OR STAXYN OR BAY38-9456 OR Vardenafil 10mg OR Vardenafil Oral Tablet OR Vinpocetine OR ITI-214 OR PF-05180999 OR PF-05180999 Tablets OR pf 05180999 OR Milrinone OR Cilostazol OR Cilostazol 100 mg OR Cilostazol 100mg OR Enoximone OR Rolipram OR Roflumilast OR Roflumilast cream OR Roflumilast Cream 0.15% OR Roflumilast 500 Mcg Oral Tablet OR sildenafil OR Sildenafil Citrate OR Sildenafil 50 mg OR sildenafil ODT OR Sildenafil 100 mg OR tadalafil OR Avanafil OR avanafil 200mg OR Avanafil 50 MG OR Avanafil 50 MGOR Avanafil 100 mg OR Avanafil 200 mg OR udenafil OR Udenafil OR Zydene OR Udenafil 50 mg OR Udenafil 150 mg OR Udenafil 100 mg OR TP10 OR Doxofylline OR Ibudilast OR Ibudilast 50mg OR Ibudilast 20mg OR Apremilast OR Apremilast 30mg OR Apremilast 20mg OR Apremilast OR CC-10004 OR Apremilast 30 mg OR OSI-461 OR Mirodenafil OR Mirodenafil 100mg OR Mirodenafil 50mg tablet OR PF-04447943 OR PF-04447943 25 mg OR Vesnarinone OR GSK256066 OR gsk 655066 OR MK-0873 OR MK-0873 Patch OR MK-0873 Cream OR MK-0873 2.5 mg OR MK-0873 1.25 mg OR E6005 OR E6005 0.2% OR E6005 0.05% OR Caffeine OR theophylline OR Theophylline ER OR Theophylline 300 mg OR theophylline SR OR Theophylline 300mg OR Pentoxifylline OR Pentoxifylline 400 MG OR Pentoxifylline OR PTX OR Pentoxifylline Oral Tablets OR Levosimendan OR Levosimendan 2.5 mg/ml Injectable Solution OR levosimendan at a dose of 6 mcg/kg OR Levosimendan infusion OR Levosimendan 2.5 MG/ML OR Dipyridamole OR Dipyridamole 75 MG OR Dipyridamole 200 mg OR Dipyridamole Tablets OR Dipyridamole 25Mg Tab OR Papaverine OR Papaverine Hydrochloride OR Aminophylline OR Aminophylline group OR aminophylline bolus OR Aminophylline 400 mg OR aminophylline hydrate OR cilomilast OR Anagrelide OR anagrelide hydrochloride OR Anagrelide retard OR Anagrelide Hydrochloride Capsules 1 mg OR Anagrelide CR OR Crisaborole OR Crisaborole ointment 2% OR Crisaborole Ointment OR Crisaborole 2% Top Oint OR Drotaverine hydrochloride OR zatolmilast OR Zatoimilast/ BPN14770 OR Ensifentrine OR Ensifentrine 3mg OR Ensifentrine Dose 1 OR Ensifentrine OR RPL554 suspension

14 results were returned of which only 4 were relevant studies to rhythm control. Of these 4 only 2 were completed. 1 of these looked at the effect of caffeine on the development of post-operative AF (NCT01999829) while another looked at the influence of caffeine on (NCT02095405) patients with cardiac arrhythmias. Neither found significant effects (1, 2). Moreover, caffeine is a promiscuous compound with multiple targets (phosphodiesterases, adenosine receptors, ryanodine receptors) in multiple cell types therefore these studies do not provide any results or evidence that can tell us something about the role of phosphodiesterases in atrial myocytes in atrial fibrillation. Since searches using the condition/disease filter are reported to return less results than other filters we performed another search:

Other terms: Atrial Flutter OR Atrial Fibrillation | Intervention/ treatment:

Phosphodiesterase Inhibitors OR phosphodiesterase type 5 inhibitor OR Phosphodiesterase 5 Inhibitor OR Phosphodiesterase type 5 Inhibitor OR PDE5 OR PDE5 inhibitor OR PDE5i arm OR PDE5 Inhibitor OR PDE5- inhibitors group OR PDE5 inhibitor OR tadalafil OR PDE9i OR Vardenafil OR Levitra OR BAY38-9456 OR Vardenafil ODT OR STAXYN OR BAY38-9456 OR Vardenafil 10mg OR Vardenafil Oral Tablet OR Vinpocetine OR ITI-214 OR PF-05180999 OR PF-05180999 Tablets OR pf 05180999 OR Milrinone OR Cilostazol OR Cilostazol 100 mg OR Cilostazol 100mg OR Enoximone OR Rolipram OR Roflumilast OR

Roflumilast cream OR Roflumilast Cream 0.15% OR Roflumilast 500 Mcg Oral Tablet OR sildenafil OR Sildenafil Citrate OR Sildenafil 50 mg OR sildenafil ODT OR Sildenafil 100 mg OR tadalafil OR Avanafil OR avanafil 200mg OR Avanafil 50 MG OR Avanafil 50 MGOR Avanafil 100 mg OR Avanafil 200 mg OR udenafil OR Udenafil OR Zydene OR Udenafil 50 mg OR Udenafil 150 mg OR Udenafil 100 mg OR TP10 OR Doxofylline OR Ibudilast OR Ibudilast 50mg OR Ibudilast 20mg OR Apremilast OR Apremilast 30mg OR Apremilast 20mg OR Apremilast OR CC-10004 OR Apremilast 30 mg OR OSI-461 OR Mirodenafil OR Mirodenafil 100mg OR Mirodenafil 50mg tablet OR PF-04447943 OR PF-04447943 25 mg OR Vesnarinone OR GSK256066 OR gsk 655066 OR MK-0873 OR MK-0873 Patch OR MK-0873 Cream OR MK-0873 2.5 mg OR MK-0873 1.25 mg OR E6005 OR E6005 0.2% OR E6005 0.05% OR Caffeine OR theophylline OR Theophylline ER OR Theophylline 300 mg OR theophylline SR OR Theophylline 300mg OR Pentoxifylline OR Pentoxifylline 400 MG OR Pentoxifylline OR PTX OR Pentoxifylline Oral Tablets OR Levosimendan OR Levosimendan 2.5 mg/ml Injectable Solution OR levosimendan at a dose of 6 mcg/kg OR Levosimendan infusion OR Levosimendan 2.5 MG/ML OR Dipyridamole OR Dipyridamole 75 MG OR Dipyridamole 200 mg OR Dipyridamole Tablets OR Dipyridamole 25Mg Tab OR Papaverine OR Papaverine Hydrochloride OR Aminophylline OR Aminophylline group OR aminophylline bolus OR Aminophylline 400 mg OR aminophylline hydrate OR cilomilast OR Anagrelide OR anagrelide hydrochloride OR Anagrelide retard OR Anagrelide Hydrochloride Capsules 1 mg OR Anagrelide CR OR Crisaborole OR Crisaborole ointment 2% OR Crisaborole Ointment OR Crisaborole 2% Top Oint OR Drotaverine hydrochloride OR zatolmilast OR Zatoimilast/ BPN14770 OR Ensifentrine OR Ensifentrine 3mg OR **Ensifentrine Dose 1 OR Ensifentrine OR RPL554 suspension.** This returned 26 results. 7 are still looking for participants and none of these are looking at the effect of a specific phosphodiesterase inhibitor on the incidence of atrial fibrillation. Of the 19 remaining 12 were either not relevant on closer inspection or had already been returned by the 1st search. In study, NCT01467661 looked at the long-term treatment of patients with Anagrelide hydrochloride (a PDE3 inhibitor and phospholipase A2 inhibitor) 1/52 patients developed atrial fibrillation, 3/52 had supraventricular extrasystoles, 1/52 had supraventricular tachycardia this was a phase 3b trial and anagrelide was not compared to placebo. Therefore, it is unclear if these cardiac arrhythmias are caused by the drug or features of the patients. In a placebo-controlled study in healthy patients anagrelide hydrochloride increased heart rate however no atrial arrhythmias were reported within 12 hours of administration (3). Trial **NCT01188369** was terminated prematurely because of a high incidence of post-operative AF. It is unclear if Levosimendan (PDE3 inhibitor, Ca²⁺ sensitizer and K⁺ channel activator) lead to an increased incidence of post-operative atrial fibrillation or whether it was a feature of the population. Several other studies looked at levosimendan **NCT01969071, NCT04573049**. From a meta-analysis study levosimendan appears to increase the incidence of pre-operative AF but decrease the incidence of post-operative AF (4, 5). It is unclear how levosimendan is having these effect and whether the difference reflects difference in the populations who undergo surgery or not. However, levosimendan is considered to have a pleotropic nature (6) highlighting the futility of attempting to use this data to understand the potential of phosphodiesterases as treatments for atrial fibrillation. Study NCT03671759 looked at the effect of coffee on atrial and ventricular ectopy (7). The effects of this study are again impossible to interpret as evidence for the role of PDEs in the atria or atrial fibrillation due to the multiple different components of coffee.

We performed another search of Pubmed human clinical trials and case reports using the following search:

((Atrial Fibrillation) OR (Atrial Fibrillation) OR (AF) OR (Atrial Fibrillation Persistent) OR (Atrial Fibrillation New Onset) OR (Atrial Fibrillation Paroxysmal) OR (Atrial Flutter) OR (Atrial Tachycardia)) AND ((Phosphodiesterase Inhibit) OR (PDEs) OR (phosphodiesterase type 5 inhibit) OR (Phosphodiesterase 5 Inhibit) OR (Phosphodiesterase type 5 Inhibit) OR (PDE5) OR (PDE5 inhibit) OR (PDE5i arm) OR (PDE5 Inhibit) OR (PDE5- inhibit) OR (s group) OR (PDE5 inhibit) OR (tadalafil) OR (PDE9i) OR (Vardenafil) OR (Levitra) OR (BAY38-9456) OR (Vardenafil ODT) OR (STAXYN) OR (BAY38-9456) OR (Vardenafil 10mg) OR (Vardenafil) OR (al Tablet) OR (Vinpocetine) OR (ITI-214) OR (PF-05180999) OR (PF-05180999 Tablets) OR (pf 05180999) OR (Milrinone) OR (Cilostazol) OR (Cilostazol 100 mg) OR (Cilostazol 100mg) OR (Enoximone) OR (Rolipram) OR (Roflumilast) OR (Rofluminlast cream) OR (Roflumilast Cream 0.15%) OR (Roflumilast 500 Mcg) OR (al Tablet) OR (sildenafil) OR (Sildenafil Citrate) OR (Sildenafil 50 mg) OR (sildenafil ODT) OR (Sildenafil 100 mg) OR (tadalafil) OR (Avanafil) OR (avanafil 200mg) OR (Avanafil 50 MG) OR (Avanafil 50 MG) OR (Avanafil 100 mg) OR (Avanafil 200 mg) OR (udenafil) OR (Udenafil) OR (Zydena) OR (Udenafil 50 mg) OR (Udenafil 150 mg) OR (Udenafil 100 mg) OR (TP10) OR (Doxofylline) OR (Ibudilast) OR (Ibudilast 50mg) OR (Ibudilast 20mg) OR (Apremilast) OR (Apremilast 30mg) OR (Apremilast 20mg) OR (Apremilast) OR (CC-10004) OR (Apremilast 30 mg) OR (OSI-461) OR (Mirodenafil) OR (Mirodenafil 100mg) OR (Mirodenafil 50mg tablet) OR (PF-04447943) OR (PF-04447943 25 mg) OR (Vesnarinone) OR (GSK256066) OR (gsk 655066) OR (MK-0873) OR (MK-0873 Patch) OR (MK-0873 Cream) OR (MK-0873 2.5 mg) OR (MK-0873 1.25 mg) OR (E6005) OR (E6005 0.2%) OR (E6005 0.05%) OR (Caffeine) OR (theophylline) OR (Theophylline ER) OR (Theophylline 300 mg) OR (theophylline SR) OR (Theophylline 300mg) OR (Pentoxifylline) OR (Pentoxifylline 400 MG) OR (Pentoxifylline) OR (PTX) OR (Pentoxifylline) OR (al Tablets) OR (Levosimendan) OR (Levosimendan 2.5 mg/ml Injectable Solution) OR (levosimendan at a dose of 6 mcg/kg) OR (Levosimendan infusion) OR (Levosimendan 2.5 MG/ML) OR (Dipyridamole) OR (Dipyridamole 75 MG) OR (Dipyridamole 200 mg) OR (Dipyridamole Tablets) OR (Dipyridamole 25Mg Tab) OR (Papaverine) OR (Papaverine Hydrochl) OR (ide) OR (Aminophylline) OR (Aminophylline group) OR (aminophylline bolus) OR (Aminophylline 400 mg) OR (aminophylline hydrate) OR (cilomilast) OR (Anagrelide) OR (anagrelide hydrochl) OR (ide) OR (Anagrelide retard) OR (Anagrelide Hydrochl) OR (ide Capsules 1 mg) OR (Anagrelide CR) OR (Crisab) OR (ole) OR (Crisab) OR (ole ointment 2%) OR (Crisab) OR (ole Ointment) OR (Crisab) OR (ole 2% Top Oint) OR (Drotaverine hydrochl) OR (ide) OR (zatolmilast) OR (Zatolmilast/ BPN14770) OR (Ensifentrine) OR (Ensifentrine 3mg) OR (Ensifentrine Dose 1) OR (Ensifentrine) OR (RPL554 suspension)).

As well as the studies discussed in the main body of the review, this search also identified a few studies or case reports that investigated the effect of theophylline (8, 9) or dipyridamole (10) on atrial arrhythmias. However, both these drugs have multiple targets (11, 12). For example, theophylline as well as inhibiting phosphodiesterase is a known antagonist of adenosine receptors (11). Therefore, the utility of such findings to reveal the role of PDEs in AF is questionable.

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