

The Interplay Between Molecular Architecture, Pharmacology, and Suspected Adverse Drug Reactions Associated with Non-Steroidal Androgen Antagonists in The United Kingdom

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Abstract

Aims. To correlate potential links between the suspected adverse drug reaction (ADR) profile of licensed non-steroidal androgen receptor antagonists (NSARA) with their unique chemical properties and known off-target polypharmacology.

Methods. Physiochemical and polypharmacology data was curated from the Electronic Medicines Compendium, FDA New Drug Applications documents, and ChEMBL databases. System organ class (SOC, MedDRA) suspected ADRs and fatalities were curated from the United Kingdom Medicines and Healthcare products Regulatory Authority (MHRA) Yellow card spontaneous reporting scheme for their respective prescribing period; apalutamide (Jan 2019-), bicalutamide (Aug 2018-), enzalutamide (Aug 2018-), flutamide (Aug 2018-) and darolutamide (March 2019-) until Oct 2023. The number of daily doses (*dd*) was extracted from OpenPrescribing and NHS Digital secondary care medicines data. Data was standardised before comparison to suspected ADRs and fatality reports per 100,000 *dd*.

Results. A total of $n = 2,480$ suspected ADRs were associated with 42,903,000 *dd* of NSARAs in the United Kingdom. The highest number of ADRs were associated with enzalutamide ($n = 1,091$) and bicalutamide ($n = 749$). Enzalutamide was found to have the most off-target pharmacological interactions of the NSARAs studied ($n = 5$) including potent inhibition of γ -aminobutyric acid, GABA receptor ($IC_{50} = 2.6 \mu M$ vs $C_{max} = 7.7 \mu M$) associated with nervous system disorders ($n = 72$, accounting for 73% of all NSARA ADRs in this SOC). Apalutamide, the only other GABA inhibitor ($IC_{50} = 3 \mu M$ vs $C_{max} = 2.9 \mu M$) had the highest relative rate of suspected nervous system ADRs at 1.08 per 100,000 *dd*.

Apalutamide was also a modest inhibitor of the human Ether-à-go-go-Related Gene (hERG) ion channel ($IC_{50} = 6 \mu M$ vs $C_{max} = 2.9 \mu M$) and had the highest rate of suspected cardiac arrhythmia ADRs, 30-fold over, enzalutamide, a significantly weaker hERG inhibitor ($15.7 \mu M$ vs $C_{max} = 7.7 \mu M$).

Darolutamide was the only NSARA to show effects at 5-HT (serotonin) receptor at $< 10 \mu M$ but did not translate to psychiatric disorders due to low clinical BBB penetration but an association with hepatobiliary and cardiac disorders was identified based on this inhibitory axis. Suspected skin and subcutaneous SOC ADRs was associated with all NSARAs (except flutamide) but did not reach statistical significance ($P = .25$). A rationale for epidermis reactions relating to apalutamide containing a masked arylamine was explored but molecular matched pair (MMP) analysis with enzalutamide suggests it may not be a chemical cause. Statistical significance ($P < .05$) was identified in reported fatalities associated with NSARAs, flutamide had $n = 24$ or 897.5 fatalities per 100,000 *dd* which was likely due to both the indication and the small number of *dd* ($n = 3,000$) during the time period of the study.

NOTE: This preprint reports new research that has not been certified by peer review and should not be used to guide clinical practice.

Conclusions. An investigation of suspected ADRs, standardised to the number of *dd* for the novel NSARA drug class identified SOCs of potential interest. The highest number of reports related to enzalutamide and bicalutamide. Suspected skin and subcutaneous ADRs approached statistical significance and was interrogated for chemical and pharmacological connections for the first time with the aid of MMP analysis. A potential correlation to nervous system disorders and cardiac arrhythmia for the GABA and hERG inhibitors, enzalutamide and apalutamide, respectively was identified. Darolutamide's interaction with 5-HT may influence ADRs associated with cardiac and hepatobiliary SOCs. Statistically significant number of suspected fatalities with flutamide was identified.

Keywords

Prostate cancer, androgen receptor antagonist, side effect profile, polypharmacology, pharmacovigilance, flutamide, bicalutamide, darolutamide, apalutamide, enzalutamide.

Introduction

In the United Kingdom, 52,300 men per year are diagnosed with prostate cancer, [1] making it the most common cancer with peak diagnosis between 70-74 years old. [1] Hormone therapy is recommended as the first-line treatment strategy by the National Institute for Health and Care Excellence (NICE). [2-3] Androgen receptor antagonists (ARA) inhibit the exertion of androgen-like testosterone and dihydrotestosterone (DHT) pharmacological effects by competitively inhibiting testosterone binding to ARs [4]. Steroidal ARAs treat a range of conditions including sexual deviation and hot flushes with gonadorelin analogue therapy. [5]

Non-steroidal ARAs (NSARAs) are specifically used for the treatment of prostate cancer. NSARAs target prostate cancer cells by inhibiting androgen production, therefore, decreasing prostate cancer cell growth. [6] NSARAs can be either first generation (bicalutamide, flutamide) which exclusively target AR translocation to the nucleus or second generation (enzalutamide, apalutamide, darolutamide) which have further improvements on this mechanism of action (MoA). [7-8] Monitoring adverse drug reactions (ADRs) reported to the MHRA Yellow Card Scheme in the United Kingdom [9] is important as 1 in 16 patients admitted to hospital are suspected to be experiencing an ADR [10]. The ability to determine correlation of NSARAs ADRs to molecular structure and/or off-target pharmacological factors is therefore timely. [11-18]

Methods

The licensed first generation NSARAs are bicalutamide [19] and flutamide [20]; and second generation, enzalutamide [21], apalutamide [22], darolutamide [23] (**Figure 1**) were selected based on inclusion and exclusion criteria of this study (**Table 1**).

Table 1. Inclusion and exclusion criteria

Inclusion criteria	Exclusion criteria
ARA type:	ARA type:
Licensed Non-steroidal ARA – AR antagonists (first and second generation)	Steroidal ARA and non-steroidal ARA – androgen synthesis inhibitors.
	Non licensed non-steroidal ARA (AR antagonist) in the UK
Prescribing data in the UK (primary care and secondary care).	Non-steroidal ARA with no prescribing data in the UK.

Available yellow card ADR reporting during No ADR reports on Yellow Card Scheme prescribing data window.

Known age ranges for ADR reports.

Unknown age reports.

Prescribing Data

NSARA data prescribing information was curated from OpenPrescribing database (for primary care prescribing [24]) and NHS Digital Secondary Care Medicines Data. [25] These datasets provided the tablet quantities of the NSARAs dispensed from each NHS trust.

Drug pharmacology

NSARA's molecular and physiochemical properties were extracted from the Chemical database of bioactive molecules with drug-like properties, European Molecular Biology Laboratory (ChEMBL), [26] FDA New Drug Application (NDA) documents, [27-30] and the Electronics Medicines Compendium (EMC). [31] Dosing regimens were extracted from the British National Formulary (BNF). [32]

The following parameters were calculated: pIC_{50} was calculated from the negative log of the average AR IC_{50} data. Lipophilic ligand efficiency (LLE) was calculated using the following equation: $pIC_{50} - \log_{10}P$. [33]. The predicted blood-brain barrier (BBB) penetration requirements were interrogated as follows: molecular weight (MW) <450 Da, <6 hydrogen bond donors (HBD), <2 hydrogen bond acceptor (HBA), a neutral or basic drug (indicated by pK_a), topological polar surface area (tPSA) <90Å, $\log_{10}D$ (at pH 7.4) between 1 to 3 and a low p-glycoprotein affinity. The C_{max} was converted from ng/mL to nM based on the unique molecular weight of each NSARA.

Adverse drug reaction reporting

Suspected adverse drug reactions (ADRs) of all United Kingdom licensed NSARAs were extracted from the MHRA Yellow Card interactive drug analysis profile (iDAPs). [9] Suspected ADRs reported for apalutamide (since Jan 2019), bicalutamide (since Aug 2018), enzalutamide (since Aug 2018), flutamide (since Aug 2018), and darolutamide (since Mar 2019) with data extracted corresponding to the longitudinal timeline of each NSARAs prescribing in the United Kingdom.

Standardised NSARA ADRs were determined by calculating the ADR incidence rate per 100,000 daily doses (dd) for each drug:

1. Total tablets quantity dispensed divided by the respective standard of NSARA daily number of tablets = total dd .
2. Scale factor: Total dd dispensed / 100,000.
3. ADRs multiplied by the scale factor gave the ADRs per 100,000 dd of the NSARAs.

Target Affinity

ChEMBL was used to extract the target proteins for each NSARA (accessed 29th November 2023). [26] Where > 1 IC_{50} for a human protein target was available, the mean IC_{50} value was calculated.

Ethical Approval

Ethical approval was not required by the School of Pharmacy sub-ethics committee due to the use of fully anonymised patient data.

Statistical Analysis

Excel for Microsoft 365 was used to perform chi-squared (χ^2) tests on the standardised ADR's/100,000 dd data. A $P < .05$ was set for statistical significance.

Results

Physicochemical properties and pharmacokinetics

Properties of the NSARAs ($\log_{10}P$, pIC_{50} , LLE) are shown in **Table 2**. Enzalutamide was the most lipophilic drug ($\log_{10}P = 3.99$) and darolutamide was the most potent AR antagonist ($pIC_{50} =$

7.59). All AR antagonists had an LLE < 5. NSARAs that comply with the BBB criteria: bicalutamide, flutamide and darolutamide successfully passed the MW requirements; with apalutamide being the heaviest drug (477 Da). All the NSARA's failed the guidelines for number of HBAs by having >2 HBA's, however, passed the HBD criteria (<6). Apalutamide, enzalutamide and darolutamide are weak bases and bicalutamide and flutamide are neutral. Apalutamide, enzalutamide and flutamide had a $\text{pK}_a < 9.0$. Bicalutamide and darolutamide met the criteria for $\log_{10}D^{7.4}$ (2.71 and 2.44, respectively). Enzalutamide and flutamide were not P-glycoprotein substrates. Overall, flutamide passed the most BBB requirements out of all AR antagonists (achieving 5/7 BBB requirements), whilst apalutamide met the least (3/7 conditions).

In clinical settings, darolutamide did not significantly alter cerebral blood flow (CBF), consistent with its low clinical BBB penetration and subsequent low risk of CNS-related adverse events. [34] However, a significant reduction in CBF was observed with enzalutamide suggestive of increased BBB penetration. [34] This was further supported by cerebrospinal fluid (CSF) for enzalutamide in rats showing a 3-6% penetration. [35] CSF measurement in dogs for apalutamide show a 2-5% penetration, [36] A single case report for CSF leak with flutamide was identified [37] but no clinical data was identified for bicalutamide.

Flutamide required the most daily tablets; with the regime consisting of two tablets, three times a day due to the short half-life (10 h). Apalutamide had the largest volume of distribution (276 L/kg) with bicalutamide and flutamide having undetermined volumes of distribution (V_d).

Target affinity

Table 3 shows the strength of inhibition assessed by using the mean IC_{50} values. Apalutamide and darolutamide had the most potent inhibition of the AR receptors, with $IC_{50} = 63$ nM and 26 nM, respectively. Bicalutamide and flutamide had the weakest AR antagonist inhibition (1.2 and 1.3 μM , respectively). Bicalutamide had additional interactions with progesterone receptors at clinically achievable levels ($IC_{50} = 1.8$ μM vs $C_{\text{max}} = 1.7$ μM). Enzalutamide was found to have the most off-target pharmacological interactions of the NSARAs studied ($n = 5$) including potent inhibition of γ -aminobutyric acid, GABA receptor ($IC_{50} = 2.6$ μM vs $C_{\text{max}} = 7.7$ μM). Apalutamide, was the only other GABA inhibitor ($IC_{50} = 3$ μM vs $C_{\text{max}} = 2.9$ μM) and a modest inhibitor of the human Ether-à-go-go-Related Gene (hERG) ion channel ($IC_{50} = 6$ μM vs $C_{\text{max}} = 2.9$ μM). Enzalutamide was a significantly weaker hERG inhibitor (15.7 μM vs $C_{\text{max}} = 7.7$ μM). Furthermore, darolutamide was the only NSARA to show effects at 5-HT (serotonin) receptor at < 10 μM .

Total ADR's and fatalities

ADR profiles of the NSARAs were standardised based on the number of *dd* dispensed in the time period of the study (**Table 4**). Overall, flutamide had the largest number of ADR's/100,000 *dd* (15.8); followed by darolutamide (15.0) with enzalutamide the least (3.1). Flutamide had the largest number of fatalities/100,000 *dd* ($n = 24$ (897.5 per 100,000 *dd*)). Bicalutamide had the next highest at 0.4 deaths per 100,000 *dd*. Darolutamide was the only NSARA to have no reported deaths. For the selected SOC based on relative proportions of suspected ADRs and > 100,000 *dd* during the time period are shown in **Figure 2**.

Blood and lymphatic system disorder ADR's and fatalities

Darolutamide and apalutamide had the highest reports of 0.44 and 0.24 ADRs per 100,000 *dd*, respectively. Patients taking darolutamide experienced suspected neutropenia (0.44/100,000 *dd*) and apalutamide experiencing suspected thrombocytopenia (0.12/100,000 *dd*).

Cardiac disorders ADR's and fatalities

Darolutamide had the highest suspected cardiac disorder profile (0.89/100,000 *dd*), apalutamide had the second highest ADRs/100,000 *dd* (0.78), along with being the only AR antagonist to have a suspected fatality in this category. The main cardiac event possessed by apalutamide patients were cardiac arrhythmias and heart failure, 0.3 and 0.36 per 100,000 *dd* respectively. Whilst enzalutamide exhibited the least cardiac events ($n = 12$, 0.03 per 100,000 *dd*).

General disorders and administration site conditions ADR's and fatalities

General disorders and administration site conditions had the highest number of ADRs across all AR antagonists. Enzalutamide had a fatality rate of 0.8 per 100,000 *dd*. Apalutamide also experienced a high prevalence of general disorders side effects (2.04/100,000 *dd*) and fatalities (0.06/100,000 *dd*). Darolutamide followed with 1.78 ADRs/100,000 *dd*.

Nervous system ADR's and fatalities

Similarly, to general disorder ADR's, apalutamide (1.08/100,000 *dd*) and darolutamide (0.44/100,000 *dd*) had the highest nervous system ADRs. Apalutamide's main nervous system ADR was neurological disorders (0.48/100,000 *dd*).

Psychiatric disorders ADR's and fatalities

Darolutamide had 0.44 ADRs per 100,000 *dd*, which was the highest number of suspected events for the NSARA series. Patients on enzalutamide experienced 0.1 suspected psychiatric reactions /100,000 *dd*.

Hepatobiliary disorders ADR's and fatalities

The highest suspected ADRs of patients with hepatobiliary disorders were experience with flutamide (37.4 per 100,000 *dd*) but may be an artefact of the very low number of *dd* in this time period.

Skin and subcutaneous tissue disorder ADR's and fatalities

Similar to general disorders, skin and subcutaneous disorders had the largest number of suspected ADRs/100,000 *dd*. Darolutamide (2.67/100,000 *dd*) and apalutamide (2.4/100,000 *dd*) had the vast number of ADRs, with all darolutamide patients experiencing epidermal ADR's and 2.16 ADR's/100,000 *dd* for apalutamide.

Age range of NSARA ADR reports

Apalutamide consisted of 72 patients who experienced a suspected ADR between the ages of 50-89 years old. Bicalutamide had 2 patients between 0-19 years old, with the remaining 35 patients between 60-99 years old. Enzalutamide consisted of 140 patients with side effects between 50-99 years old. Flutamide's single report from the selected SOCs was from a male patient that was between 80-89 years old; and darolutamide having 14 patients between the ages of 60-89.

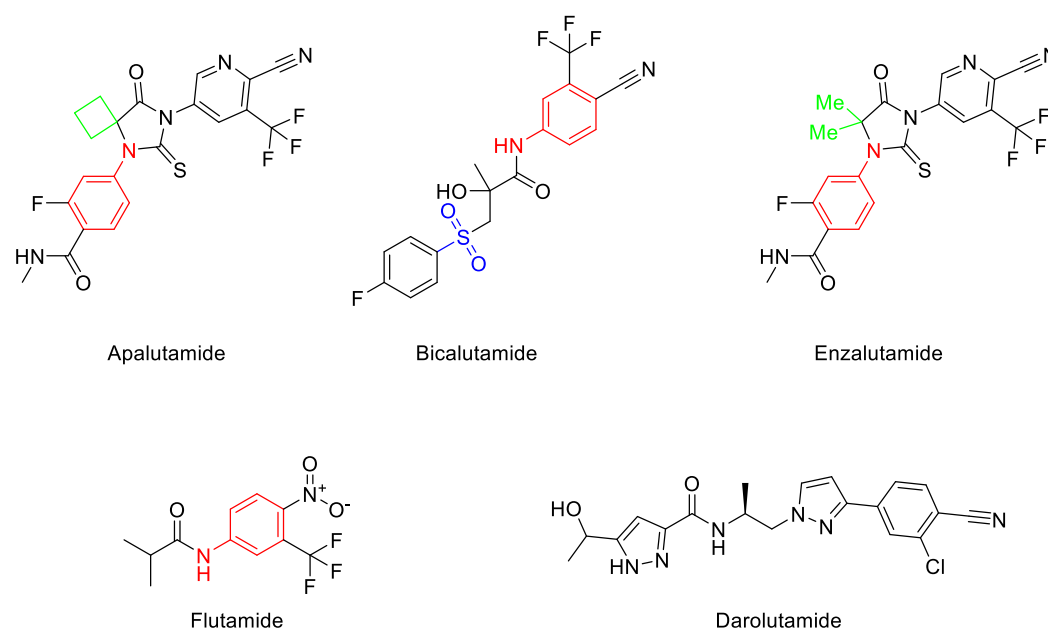


Figure 1. Structures of NSARAs studied. Colour coding: red for masked arylamine motif; blue for sulfonyl group; green for additional MMP analysis.

Molecular matched pair (MMP) analysis was possible between apalutamide versus enzalutamide (**Figure 1**) due to a single point variation present - gem-dimethyl versus *spirocyclobutane*, respectively on the thiohydantoin scaffold. This led to a logarithmic difference in activity at the AR (63 vs 806 nM), and a 2.5-fold difference in hERG activity (6 vs 15.7 μ M), comparable inhibition at the GABA receptor, and weak inhibition at glucocorticoid and progesterone receptors, respectively.

Variable	Apalutamide		Bicalutamide		Enzalutamide		Flutamide		Darolutamide
Molecular obesity and on-target efficiency metrics									
clog ₁₀ P	3.53		2.88		3.99		3.21		2.67
AR pIC ₅₀	7.09		4.99		5.74		4.48		7.59
LLE	3.58		2.11		1.75		1.27		4.92
Blood-brain barrier penetrant properties									
MW (Da)	477.44		430.38		464.44		276.21		398.85
pK _a	13.05		11.78		13.05		12.81		9.81
^t PSA (Å)	89.33		107.26		76.44		72.24		119.62
HB acceptors	5		4		4		3		4
HB donors	1		2		1		1		3
Clog ₁₀ D _{7.4}	3.46		2.71		4.16		3.27		2.44
P-glycoprotein substrate	Yes		Yes		No		No		Yes
No. of BBB requirements met	3		4		4		5		4
Pharmacokinetics									
Bioavailability (F, %)	100		-		84.2		-		30
t _½ (h)	72		168		139.2		10		20
T _{max} (h)	2		19	3	2		1.71	2.21	4
C _{max} (nM) (2dp)	2864.64 [A]	2816.90 [NDA]	1705.47 ((R)-enantiomer)	195.18 ((S)-enantiomer)	7709.7 [E]	5898.39 [NDE]	- [F]	- [H]	529.72
Hepatic metabolism	Yes: CYP-2C8, -2C9, -2C19, -3A4, [C]		Yes: CYP3A4	Yes: Isoenzyme & glucuronidation	Yes: CYP-2C8, -2C9, -2C19, -3A4,	Yes: [C] 1	Yes: CYP-1A2	-	Yes: CYP3A4
Renal Excretion (%)	65		50		71		45		63.4
Volume of distribution (L/kg)	276		-		110		-		119
Clearance (L/h)	1.3		0.32		0.542		-		116
PPB	96% [A]	95% [NDA]	99% ((R)-enantiomer)	96% ((S)-enantiomer)	97.5% [E]	95% [NDE]	95% [F]	93% [H]	92%
Dosing	OD (240mg)		OD (50mg) or OD (150mg)		OD (160mg)		TDS 2x (125mg)		BD 2 x (300mg)

Table 2. Summary of the physicochemical, blood-brain barrier predicted penetration, and pharmacological properties of the five NSARAs. Colour coded green met the BBB thresholds. Abbreviations: [A], apalutamide; [NDA], *N*-desmethyl apalutamide; [E], enzalutamide; [NDE], *N*-desmethyl enzalutamide; [F], flutamide; [H], hydroxyflutamide; [C], carboxylesterases ;clog₁₀P, calculated by log₁₀P; LLE, lipophilic ligand efficiency; MW, molecular weight; pK_a, acid dissociation constant; ^tPSA, total polar surface area; HB, hydrogen bond; clog₁₀D^{7.4}; calculated log₁₀D at pH 7.4; BBB, blood-brain barrier; C_{max}, peak serum concentration; T_{max}, time taken to reach C_{max}; PPB, plasma protein binding.

Table 3. Target pharmacology (mean IC₅₀ (nM)) of the five NSARAs studied alongside C_{max} and clinical measurements for BBB penetration. n.r. = not reported.

	Apalutamide	Bicalutamide	Enzalutamide	Flutamide	Darolutamide
AR	63	1167	806	1348	26
Glucocorticoid receptor	-	-	<19500	-	-
Progesterone receptor	-	1819	-	-	-
Bile salts export pump	-	54450	-	79396	-
Progesterone receptor	No inhibition	-	16000	-	-
GABA	3000	-	2600	-	-
hERG	6000	-	15700	-	-
5-HT transporter	-	-	-	-	<10000
C_{max}	2865	1706	7710	n.r.	530
BBB penetration	2-5% (dog) [36]	n.r.	3-6% (rat) [35]	CSF leak case report [37]	0.3-0.6% (human) [34]
	10 – 100 nM				
	100 – 1000 nM				
	1000 – 10000 nM				
	> 10,000 nM				
-	Undetermined				

Table 4. Summary of the suspected ADRs for the five NSARAs. Key: Total ADRs calculated by total ADRs / 100,000 dd; fatalities calculated by fatalities / 100,000 dd; p-values calculated by chi-squared test; *P* values exclude flutamide (apart from fatalities) and where there are n < 3 reports.

	Apalutamide	Bicalutamide	Enzalutamide	Flutamide	Darolutamide	P
Number of dd /100,000	1700000 (17.0)	5500000 (55.0)	35500000 (355.0)	3000 (0.03)	200000 (2.0)	-
Total ADRs	182 (10.93)	749 (13.69)	1091 (3.07)	423 (15.82)	35 (15.55)	.08
Fatalities	2 (0.12)	22 (0.40)	50 (0.14)	24 (897.53)	0	< .05
Blood and lymphatic system disorders						
Total ADRs	4 (0.24)	3 (0.05)	4 (0.01)	0	1 (0.44)	.89
Fatalities	0	0	0	0	0	-
Thrombocytopenia	2 (0.12)	0	1 (0.002)	0	0	-
Neutropenia	0	0	1 (0.002)	0	1 (0.44)	-
Cardiac disorders						
Total ADRs	13 (0.78)	4 (0.07)	12 (0.03)	0	2 (0.89)	.70
Fatalities	1 (0.06)	0	0	0	0	-
Cardiac Arrhythmia's	5 (0.30)	0	4 (0.01)	0	0	-
Heart failure	6 (0.36)	0	0	0	1 (0.44)	-
General disorders and administration site conditions						
Total ADRs	34 (2.04)	17 (0.31)	121 (0.34)	0	4 (1.78)	.52
Fatalities	1 (0.06)	0	29 (0.08)	0	0	-
Nervous system disorders						
Total ADRs	18 (1.08)	17 (0.31)	72 (0.20)	0	1 (0.44)	.82
Fatalities	0	0	0	0	0	-
Neurological disorders NEC	8 (0.48)	8 (0.15)	33 (0.09)	0	1 (0.44)	
Psychiatric disorders						
Total ADRs	5 (0.30)	8 (0.15)	37 (0.10)	0	1 (0.44)	.94
Fatalities	0	0	0	0	0	-
Hepatobiliary disorder						
Total ADRs	0	5 (0.09)	2 (0.01)	1 (37.40)	1 (0.44)	-
Fatalities	0	0	0	0	0	
Skin and subcutaneous tissue disorders						
Total ADRs	40 (2.40)	18 (0.33)	16 (0.05)	0	6 (2.67)	.25
Fatalities	0	0	0	0	0	-
Epidermal	36 (2.16)	15 (0.27)	12 (0.03)	0	6 (2.67)	.25

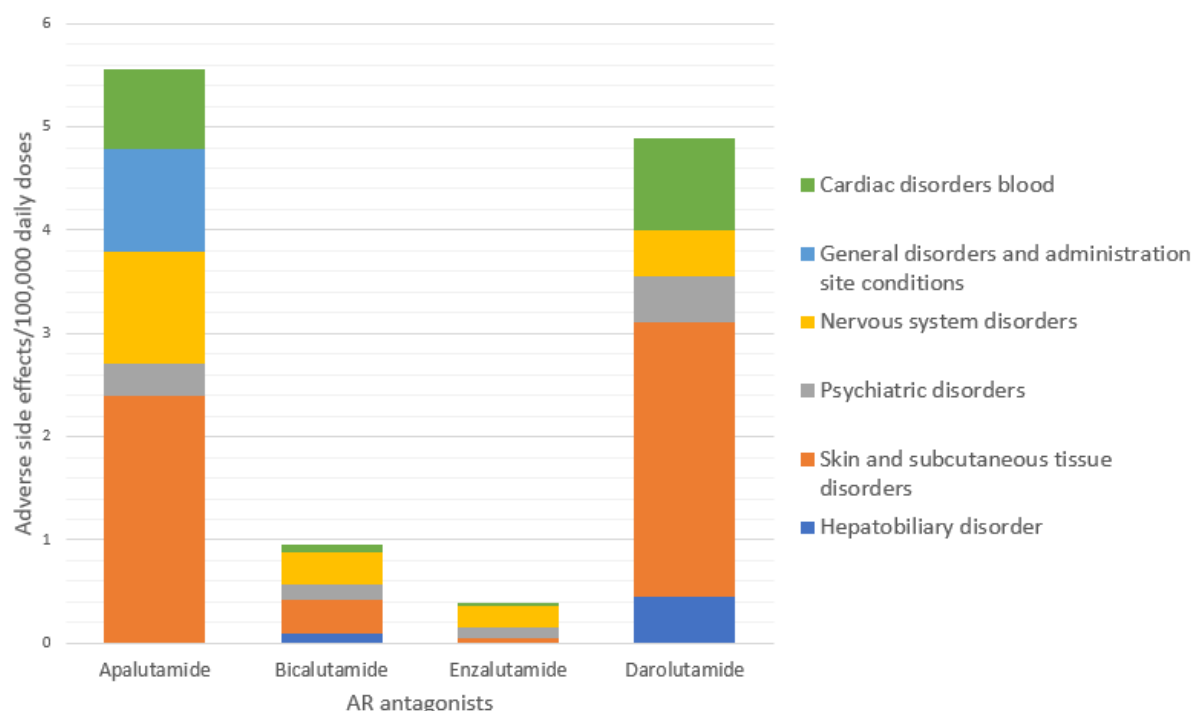


Figure 2. Bar chart comparison of the ADRs per 100,000 *dd* for the main SOC areas excluding flutamide ($n = 1$ ADR in these six major SOC's).

Discussion

Total ADR's

The highest number of total ADRs reported was with enzalutamide ($n = 1091$), when standardised to the number of *dd*, flutamide ($n = 423$, 15.82 ADRs per 100,000 *dd*) and darolutamide ($n = 35$, 15.55 ADRs per 100,000 *dd*) emerged as have a higher suspected incidence rate. Flutamide's data was excluded when comparing individual SOC ADRs per 100,000 *dd* as there was limited prescribing during the time frame of this study (3,000 *dd*) vs all other NSARAs 42,900,000 *dd*.

Total fatalities

Enzalutamide emerged as having the highest number of fatalities ($n = 50$), when standardised to the number of *dd*, flutamide ($n = 23$, 897.5 fatalities per 100,000 *dd*) emerged as having a higher fatality rate. Darolutamide had zero reported fatalities during the time period of this study.

Blood and lymphatic systems disorders ADR's and fatalities

Darolutamide had a prevalence of 0.4 ADR's/100,000 *dd* with one report of neutropenia. Apalutamide followed with 0.24 ADR's/100,000 *dd*, with reports of thrombocytopenia. Interactions between apalutamide and CYP2C8 and CYP2C9 can cause drug-drug interactions (DDI),. Enzalutamide patients experiencing both (immune thrombocytopenia and neutropenia) again possibly due to the DDI's with CYP2C8 and CYP2C9. Patients taking flutamide experienced no blood and lymphatic disorder ADR's. None of the NSARAs studied were suspected of inducing a blood and lymphatic system fatality.

Cardiac disorders ADR's and fatalities

Darolutamide had the greatest prevalence of ADRs for cardiac disorders. The increase in cardiovascular events on darolutamide could be potentially ascribed to SERT inhibition, given its importance in the cardiovascular system.[38] Furthermore, darolutamide is a known CYP3A4 inhibitor related to potential DDIs. Prostate cancer patients are within the same age group to have cardiac co-morbidities. Cardiac medications are known CYP3A4 substrates (e.g. simvastatin/ atorvastatin [39]). Additionally, p-gp modulation causes disturbances in ion efflux which can be of concern to those taking

ion channel inhibitors, such as verapamil and amiodarone. [40] P-glycoprotein inhibitors can be proton-pump inhibitors (e.g. omeprazole) and macrolides antibiotics (e.g. clarithromycin & erythromycin). The combination of these factors could have a detrimental impact on the patients' health by increasing the risk for a cardiovascular event that is highly dependent on polypharmacy in this age group.

Apalutamide was a modest inhibitor of the human Ether-à-go-go-Related Gene (hERG) ion channel ($IC_{50} = 6 \mu M$ vs $C_{max} = 2.9 \mu M$) and had the highest rate of suspected cardiac arrhythmia ADRs, 30-fold over the only other NSARA with this suspicion, enzalutamide, a significantly weaker hERG inhibitor ($15.7 \mu M$ vs $C_{max} = 7.7 \mu M$). Apalutamide (0.36 ADR's /100,000 dd) also interacts with CYP2C8, CYP2C9, CYP2C19 and P-gp. Inducing CYP2C19 can cause patients taking clopidogrel increase metabolism to its active metabolite, which has a half-life of approx. 30 minutes. This causes the therapeutic effect to diminish quickly after administration, making the patient at risk for an ischemic attack again dependent on polypharmacy confounders not available in these datasets.

Enzalutamide is also known to interact with CYP2C8, CYP2C9, CYP2C19, CYP3A4. Bicalutamide is a CYP3A4 inhibitor and p-gp inducer, cardiac events are potentially due to DDIs. Flutamide had no cardiac ADRs and no reported cytochrome P450 or p-gp interactions in contrast.

General disorders and administration site conditions ADR's and fatalities

A higher prevalence of ADR's was observed with enzalutamide, apalutamide and darolutamide for general disorders and administration site conditions. Apalutamide's interaction with CYP3A4, CYP2C19 and p-gp can induce DDI with other drugs that utilise their metabolic pathways. Darolutamide and bicalutamide affects CYP3A4 and p-gp, whilst enzalutamide interacts with CYP3A4. Enzalutamide had 0.08 fatalities/100,000 dd, followed by apalutamide (0.06).

Nervous system ADR's and fatalities

Effects of AR on CNS and cognitive function have been reported.[41] Apalutamide and darolutamide had 1.08 and 0.44 nervous system ADRs/100,000 dd, respectively. These higher reports are potentially due to their unique pharmacology. Inhibition of GABA receptor can lead to increased anxiety, stress, and fear responses and apalutamide, a GABA inhibitor ($IC_{50} = 3 \mu M$ vs $C_{max} = 2.9 \mu M$) had the highest relative rate of suspected nervous system ADRs at 1.08 per 100,000 dd. BIn clinical settings, darolutamide did not significantly alter cerebral blood flow (CBF), consistent with its low clinical and predicted BBB penetration and subsequent low risk of CNS-related adverse events. [34] In contrast, CSF measurement in dogs for apalutamide show a 2-5% penetration which may inform patient stratification. [36]

Psychiatric disorders ADR's and fatalities

Darolutamide and apalutamide led with the highest number of psychiatric disorders per 100,000 dd (0.44 and 0.33, respectively). Darolutamide was identified as the sole NSARA to show effects at 5-HT (serotonin) receptor at $< 10 \mu M$. 5-HT has a well-known role in depression, obsessive-compulsive disorder, and social phobia and there have been reports of increased depression in patients taking AR antagonists.[42] However, low BBB penetration of darolutamide made this off-target interaction an unlikely contributor to suspected psychiatric disorders reported. Similar to the nervous system side effect profile bicalutamide and enzalutamide followed with lower numbers of suspected reports, 0.15 and 0.1 ADRs per 100,000 dd.

Hepatobiliary disorders

Bicalutamide and flutamide both weakly inhibited BSEP. Chronic inhibition of BSEP can result in cholestasis due to the restriction of the bile passage. [43]

Flutamide had the largest reports at 0.44 per 100,000 dd, this could be due to being the only NSARA that was cleared by hepatic metabolism (approximately 50%) rather than renal clearance. Darolutamide followed with 0.44 ADR's /100,000 dd. Darolutamide was the only NSARA to show effects at 5-HT (serotonin) receptor at $< 10 \mu M$ and an association of inhibiting 5-HT and dysregulation of

crucial components of liver function including hepatic blood flow, innervation and wound healing was noted.[44]

Apalutamide and enzalutamide did not reveal significant reports of hepatic injuries, due to possessing similar drug chemical properties. They are cleared via the kidney with a renal excretion of 65% and 71%, respectively.

Skin and subcutaneous tissue disorder ADR's and fatalities

NSARAs exhibited suspected skin and subcutaneous tissue disorder ADRs impacting the epidermis. Apalutamide, darolutamide and bicalutamide possessed the largest number of ADR's/100,000 dd (2.40, 2.67, and 0.33, respectively). This may be due to the apalutamide containing an arylamine (**Figure 1**), which are molecular characteristics known to induce skin reactions.[45] Conversely, flutamide also contains an arylamine, but there were no reports of suspected skin or subcutaneous tissue ADRs. Bicalutamide (**Figure 1**) contains a sulfonyl-arylamine which is known to induce inflammation via IgE and IgG immune pathways.[46] Resulting in patients with a sulfonyl-arylamine allergy that are taking bicalutamide to experience epidermal side effects due to the hypersensitivity as a potential cause.

Limitations

Reports from the Yellow Card scheme are suspected reports, and therefore, no causal relationship must be proved before submitting a report. Therefore, reported ADRs in any registry may have no defined relationship to the pharmacology of the NSARAs. Under-reporting of ADRs is also a common issue with pharmacovigilance schemes. [47] Independent datasets were used for frequency of dispensed prescriptions and frequency of reported suspected ADRs; neither dataset enables the interpretation of comorbidity and co-prescription confounders, for example. This limits the assessment of causality and identification and adjustment for these confounding factors. Prostate cancer occurs in later stages of life where issues of medications from co-morbidities can arise.

Furthermore, the prescriber's choice of a NSARA may be influenced by known interactions (and contraindications) with drugs co-prescribed for comorbidities, i.e., there may be a significant bias by indication (or contraindication) as these comorbidities may affect sensitivity for ADRs. For example, certain NSARAs are currently cautioned as follows enzalutamide: seizures, encephalopathy, hypersensitivity, ischemic heart disease, falls and embryo-foetal tox; darolutamide: seizures, ischemic heart disease and embryo-foetal tox; and apalutamide: seizures, ischemic heart disease, fractures, falls and embryo-foetal tox. The study cannot adjust for these confounders. There is also a NSARA dose versus risk of association with an ADR that cannot be described within these datasets.

Conclusions

The detection of suspected ADR's of NSARAs and potential correlations to their respected polypharmacology and physiochemical properties was identified. The highest number of ADRs were associated with enzalutamide ($n = 1,091$) and bicalutamide ($n = 749$). Enzalutamide was found to have the most off-target pharmacological interactions of the NSARAs studied ($n = 5$) including potent inhibition of GABA receptor ($IC_{50} = 2.6 \mu M$ vs $C_{max} = 7.7 \mu M$) and had the most ADR reports for nervous system disorders ($n = 72$, accounting for 73% of all NSARA ADRs in this SOC). Apalutamide, the only other GABA inhibitor ($IC_{50} = 3 \mu M$ vs $C_{max} = 2.9 \mu M$) had the highest relative rate of suspected nervous system ADRs at 1.08 per 100,000 dd.

Apalutamide was a modest inhibitor of the hERG ion channel ($IC_{50} = 6 \mu M$ vs $C_{max} = 2.9 \mu M$) and had the highest rate of suspected cardiac arrhythmia ADRs, 30-fold over the only other NSARA with this suspicion, enzalutamide, a significantly weaker hERG inhibitor ($15.7 \mu M$ vs $C_{max} = 7.7 \mu M$). Darolutamide was the only NSARA to show effects at 5-HT (serotonin) receptor at $< 10 \mu M$ but did not translate to a correlation to psychiatric disorders due to low BBB penetration but may be associated with both cardiac and hepatobiliary suspected ADRs reported.

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References

- [1] Prostate Cancer Risk Factors | Prostate Cancer UK | Prostate Cancer UK [Internet]. [cited 2024 Jan 3]. Available from: <https://prostatecanceruk.org/prostate-information-and-support/risk-and-symptoms/are-you-at-risk>
- [2] Recommendations | Prostate cancer: diagnosis and management | Guidance | NICE.
- [3] CancerData [Internet]. [cited 2023 Oct 10]. Available from: <https://www.cancerdata.nhs.uk/treatments>
- [4] Bardal SK, Waechter JE, Martin DS. Endocrinology. Applied Pharmacology [Internet]. 2011 Jan 1 [cited 2023 Oct 4];143–76. Available from: <https://linkinghub.elsevier.com/retrieve/pii/B9781437703108000142>
- [5] Cyproterone acetate | Drugs | BNF | NICE [Internet]. [cited 2023 Oct 4]. Available from: <https://bnf.nice.org.uk/drugs/cyproterone-acetate/#indications-and-dose>
- [6] Nigro MC, Mollica V, Marchetti A, Cheng M, Rosellini M, Montironi R, et al. Current androgen receptor antagonists under investigation for resistant prostate cancer. Expert Rev Anticancer Ther [Internet]. 2022 [cited 2023 Oct 4];22(2):191–202. Available from: <https://pubmed.ncbi.nlm.nih.gov/34928193/>
- [7] Rice MA, Malhotra S V., Stoyanova T. Second-Generation Antiandrogens: From Discovery to Standard of Care in Castration Resistant Prostate Cancer. Front Oncol [Internet]. 2019 [cited 2023 Oct 10];9(AUG):801. Available from: <https://pmc/articles/PMC6723105/>
- [8] Chen Y, Zhou Q, Hankey W, Fang X, Yuan F. Second generation androgen receptor antagonists and challenges in prostate cancer treatment. Cell Death and Disease (2022) 13:632; <https://doi.org/10.1038/s41419-022-05084-1>
- [9] What is being reported | Making medicines and medical devices safer [Internet]. [cited 2023 Oct 4]. Available from: <https://yellowcard.mhra.gov.uk/idaps>
- [10] Pirmohamed M, James S, Meakin S, Green C, Scott AK, Walley TJ, et al. Adverse drug reactions as cause of admission to hospital: prospective analysis of 18 820 patients. BMJ [Internet]. 2004 Jul 1 [cited 2023 Oct 6];329(7456):15–9. Available from: <https://www.bmj.com/content/329/7456/15> SEE 2020 version instead.
- [11] Yousaf, H., Jones, A.M. Associations between Suspected Adverse Drug Reactions of HMG-CoA Reductase Inhibitors and Polypharmacology Using a National Registry Approach” Pharmacoeconomics, 2024, 3(3), 241-251 <https://www.mdpi.com/2813-0618/3/3/16>
- [12] Jones, L., Jones, A. M. “Suspected Adverse Drug Reactions of the Type 2 Antidiabetic Drug class Dipeptidyl-Peptidase IV inhibitors (DPP4i): Can polypharmacology help explain? Pharmacol. Res. Perspect. 2022, 10(6) e01029. <https://bpspubs.onlinelibrary.wiley.com/doi/full/10.1002/prp2.1029>
- [13] Salim, H, Jones, A.M. “Angiotensin II Receptor Blockers (ARBs) and Manufacturing Contamination: A Retrospective National Register Study into suspected associated adverse drug reactions” Brit. J. Clin. Pharmacol. 2022, 88(11), 4812-4827. Doi: 10.1111/bcp.15411 <https://bpspubs.onlinelibrary.wiley.com/doi/full/10.1111/bcp.15411>

- [14] Sandhu, D., Antolin, A.A., Cox, A. R., Jones, A.M. "Identification of different side effects between PARP inhibitors and their polypharmacological multi-target rationale". Brit. J. Clin. Pharmacol. 2022, 88, 742-752. <https://bpspubs.onlinelibrary.wiley.com/doi/10.1111/bcp.15015>
- [15] Matharu, K., Chana, K., Ferro, C., Jones, A. M. "Polypharmacology of clinical sodium glucose co-transport protein 2 inhibitors and relationship to suspected adverse drug reactions." Pharmacol. Res. Perspect. 2021, 9, e00867. <https://bpspubs.onlinelibrary.wiley.com/doi/10.1002/prp2.867>
- [16] Ferro, C. J., Solkhon, F., Jalal, Z., Al-Hamid, A. M., Jones, A. M. "Relevance of physicochemical properties and functional pharmacology data to predict the clinical safety profile of direct oral anticoagulants" Pharmacol. Res. Perspect. 2020, 8, e00603. <https://bpspubs.onlinelibrary.wiley.com/doi/10.1002/prp2.603>
- [17] Khan, M.; Hirsch, C.; Jones, A. M. Suspected Adverse Drug Reactions Associated with Leukotriene Receptor Antagonists Versus First Line Asthma Medications: A National Registry-Pharmacology Approach, medRxiv, 2024, <https://www.medrxiv.org/content/10.1101/2024.06.12.24308833v1>
- [18] Phillips, B., Evans, I., Skerrett, V., Jones, A.M.* United Kingdom National Register Study of Anti-Epileptic Medications: Suspected Foetal Congenital and Pregnancy-Associated Side Effects, MedRxiv, 2024, <https://doi.org/10.1101/2024.03.26.24304895>
- [19] Bicalutamide 50 mg film-coated tablets - Summary of Product Characteristics (SmPC) - (emc) [Internet]. [cited 2023 Oct 4]. Available from: <https://www.medicines.org.uk/emc/product/2053/smpc>
- [20] Flutamide 250 mg Tablets - Summary of Product Characteristics (SmPC) - (emc) [Internet]. [cited 2023 Oct 4]. Available from: <https://www.medicines.org.uk/emc/product/8428/smpc>
- [21] Xtandi 40 mg film coated tablets (Great Britain) - Summary of Product Characteristics (SmPC) - (emc) [Internet]. [cited 2023 Oct 4]. Available from: <https://www.medicines.org.uk/emc/product/10318/smpc>
- [22] Erleada 60 mg film coated tablets - Summary of Product Characteristics (SmPC) - (emc) [Internet]. [cited 2023 Oct 4]. Available from: <https://www.medicines.org.uk/emc/product/9832>
- [23] NUBEQA 300 mg film-coated tablets - Summary of Product Characteristics (SmPC) - (emc) [Internet]. [cited 2023 Oct 4]. Available from: <https://www.medicines.org.uk/emc/product/11324>
- [24] Home | OpenPrescribing [Internet]. [cited 2023 Oct 4]. Available from: <https://openprescribing.net/>
- [25] Secondary Care Medicines Data (SCMD) - Datasets - Open Data Portal BETA [Internet]. [cited 2023 Oct 4]. Available from: <https://opendata.nhsbsa.net/dataset/secondary-care-medicines-data>
- [26] ChEMBL Database [Internet]. [cited 2023 Oct 6]. Available from: <https://www.ebi.ac.uk/chembl/>
- [27] FDA Application number 22-310.
- [28] FDA Reference ID: 5025419
- [29] FDA Reference ID: 4974107
- [30] FDA Reference ID: 4291091
- [31] Home - electronic medicines compendium (emc) [Internet]. [cited 2023 Oct 6]. Available from: <https://www.medicines.org.uk/emc#gref>
- [32] BNF (British National Formulary) | NICE [Internet]. [cited 2023 Oct 10]. Available from: <https://bnf.nice.org.uk/>
- [33] Hevener KE, Pesavento R, Ren JH, Lee H, Ratia K, Johnson ME. Hit-to-Lead: Hit Validation and Assessment. Methods Enzymol. 2018 Jan 1; 610:265–309.

- [34] Williams SCR, Mazibuko N, O'Daly O, Zurth C, Patrick F, Kappeler C, Kuss I, Cole PE. Comparison of Cerebral Blood Flow in Regions Relevant to Cognition After Enzalutamide, Darolutamide, and Placebo in Healthy Volunteers: A Randomized Crossover Trial. *Target Oncol*. 2023 May;18(3):403-413. doi: 10.1007/s11523-023-00959-5.
- [35] Australian Public Assessment Report for Enzalutamide, Nov 2014, AusPAR Xtandi Enzalutamide Astellas Pharma Australia Pty Ltd PM-2013-01155-1-4
- [36] Australian Public Assessment Report for Apalutamide, Mar 2019, AusPAR Erlyand Apalutamide Janssen-Cilag Pty Ltd PM-2018-00489-1-4
- [37] Panda S, Phalak M, Thakar A, Dharanipathy S. Cerebrospinal Fluid Leak in Juvenile Nasopharyngeal Angiofibroma—Rare Sequelae of Flutamide-Induced Tumor Shrinkage. *World Neurosurg*. (2018) 120:78-81. <https://doi.org/10.1016/j.wneu.2018.07.288>
- [38] Ni W, Watts SW. 5-hydroxytryptamine in the cardiovascular system: focus on the serotonin transporter (SERT). *Clin Exp Pharmacol Physiol*. 2006 Jul;33(7):575-83. doi: 10.1111/j.1440-1681.2006.04410.x.
- [39] Rowan CG, Brunelli SM, Munson J, Flory J, Reese PP, Hennessy S, et al. Clinical importance of the drug interaction between statins and CYP3A4 inhibitors: a retrospective cohort study in The Health Improvement Network. *Pharmacoepidemiol Drug Saf* [Internet]. 2012 May [cited 2023 Dec 5];21(5):494. Available from: [/pmc/articles/PMC3890414/](https://pubmed.ncbi.nlm.nih.gov/21544414/)
- [40] Ledwitch K V., Roberts AG. Cardiovascular Ion Channel Inhibitor Drug-Drug Interactions with P-glycoprotein. *AAPS J* [Internet]. 2017 Mar 1 [cited 2023 Dec 5];19(2):409. Available from: [/pmc/articles/PMC6309310/](https://pubmed.ncbi.nlm.nih.gov/27344410/)
- [41] Ryan C, Wefel JS, Morgans AK. A review of prostate cancer treatment impact on the CNS and cognitive function. *Prostate Cancer and Prostatic Diseases* (2020) 23:207–219 <https://doi.org/10.1038/s41391-019-0195-5>
- [42] Nowakowska MK, Lei X, MR Wehner, PG Corm SH Giordano, KT Nead. Association of Second-generation Antiandrogens with Depression Among Patients with Prostate Cancer. *JAMA Network Open*. 2021;4(12): e2140803. doi:10.1001/jamanetworkopen.2021.40803
- [43] Velayudham LS, Farrell GC. Drug-induced cholestasis. *Expert Opin Drug Saf* [Internet]. 2003 May 1 [cited 2023 Nov 19];2(3):287–304. Available from: <https://pubmed.ncbi.nlm.nih.gov/12904107/>
- [44] Ruddell RG, Mann DA, Ramm GA. The function of serotonin within the liver. *Journal of Hepatology* 48 (2008) 666–675.
- [45] Anderson SE, Meade BJ. Potential Health Effects Associated with Dermal Exposure to Occupational Chemicals. *Environ Health Insights* [Internet]. 2014 [cited 2023 Nov 19];8(Suppl 1):51. Available from: [/pmc/articles/PMC4270264/](https://pubmed.ncbi.nlm.nih.gov/2470264/)
- [46] Sánchez-Borges M, Thong B, Blanca M, Ensina LFC, González-Díaz S, Greenberger PA, et al. Hypersensitivity reactions to non beta-lactam antimicrobial agents, a statement of the WAO special committee on drug allergy. *World Allergy Organization Journal*. 2013 Jan 1;6(1):18.
- [47] Hazell L, Shakir SAW. Under-reporting of adverse drug reactions: a systematic review. *Drug Saf* [Internet]. 2006 [cited 2024 Jan 4];29(5):385–96. Available from: <https://pubmed.ncbi.nlm.nih.gov/16689555/>