Transactions on Materials, Biotechnology and Life Sciences
ISSN: 2960-1932, eISSN: 2960-2319 | Volume 4
NSCBM 2024

Recent Advances of Drug Design Strategies for Classical KRAS Mutations

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Abstract. KRAS, one of the most common oncogenic mutations, is considered to be a potential target for design of cancer therapies. Previous studies have suggested a poor therapeutic index and lack of efficacy of drugs targeting the KRAS signalling pathway and even leading to think "undrugged". But recently, major breakthrough in KRAS was achieved in a decade and even up to hit the market which really inspired a lot. This review aims to illustrate the recent advances of drug design strategies for different types of KRAS mutations, including small molecular inhibitors, PROTAC and others.

Keywords: KRAS, mutation, drug design strategies.

1. Introduction

KRAS is one of the most common mutations of the rat sarcoma viral oncogene family (RAS) and is considered as the most common human oncogenic gene driver (**Figure 1.**) [1]. Crystal structure of RAS contains a hypervariable region (HVR) and a catalytic domain (G domain) consisted of switch I, switch II, and P loop which could bind guanine nucleotides along with activate downstream signalling [2, 3]. The mutations of KRAS determine the characteristics as well as form a tumour microenvironment (TME) leading to upregulating PD-L1 expression and immune escape [4, 5]. However, KRAS proteins was considered "undruggable" due to lack of deep hydrophobic binding sites [6]. As a therapeutic tool, currently available strategies to directly target mutant targets include specific targeting of 'on' and 'off' state (activation state or inactivation state), 'pan' KRAS inhibitor development, and KRAS degraders or PROTACs development. This review will specifically focus on the current drug design strategy of KRAS including both the achievements and drawbacks, hoping to give an overview of this field.

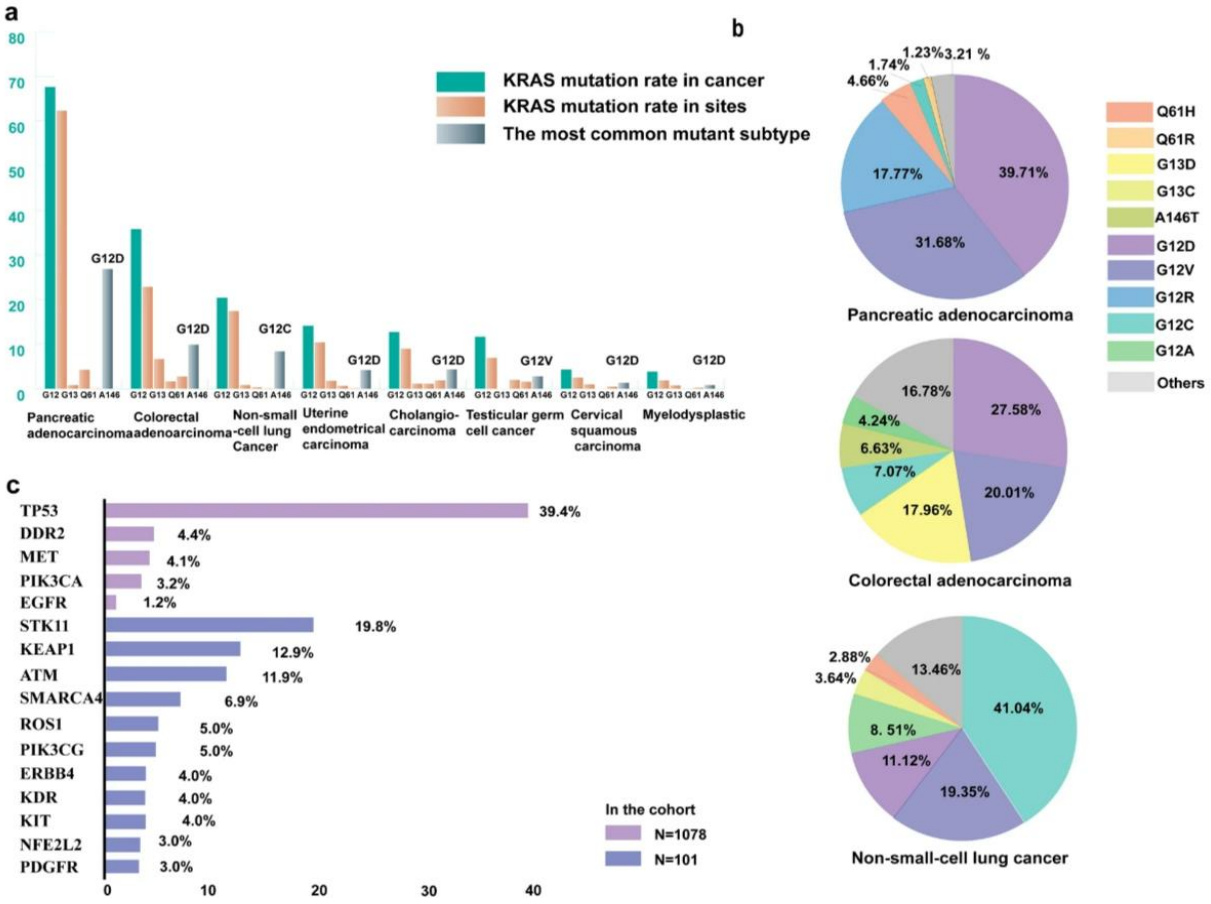


Figure 1. KRAS mutation in cancer[1].

2. Small Molecular Inhibitors

Recently, there have been surprising breakthrough highlighted by the discovery of sotorasib (**Figure 2a**) as well as adagrasib (**Figure 2b**) which are the first two KRAS inhibitors that FDA approved. These two inhibitors occupy SWIIP and modulate the cysteine nucleophilicity to capture KRAS-GDP[8, 9]. Approval of two inhibitors of KRAS G12C, Amgen's Lumakras as well as Mirati's Krazati, in 2021-2022, finally confirmed that first approved targeted therapies KRAS-mutated cancers. It should be noted that both Lumakras and Krazati target only one subtype of the G12C mutation, which affects approximately 3% of colonel cancer and 13% of NSCLC patients [10]. The 2 inhibitors currently on the market anchor the KRAS inactive state, but researchers believe that blocking KRAS active state may be more efficacious. HS-10370 (Structural formula undisclosed) is a covalent inhibition, selective, oral KRAS G12C inhibitor. In the 49 patients in the human phase I trial, the objective remission rate and DCR were 49.0% and 89.8%. [11]. Some companies anchor G12C inhibitor iterations, others target other KRAS mutation species, and others aim to minimize side effects. For clinical outcomes in different types of cancer, such as NSCLC, CRC, PDAC etc., using sotorasib and adagrasib monotherapy or multidrug combinations show different results. Compared to chemotherapy, Krazati significantly lowered the risk of tumour development or death with KRAS G12C-mutated NSCLC, meeting the primary endpoint of PFS and secondary endpoint of ORR. [12].

At the same time, KRAS p.Gly13Asp (G13D) inhibitors MRTX1133 (**Figure 2c**), ASP3082 Structural formula undisclosed), BI-2852 (**Figure 2d)**, TH-Z835 (**Figure 2e**) had entered phase I clinical trial and also expected to be promising[13-16]. For the development of activated state inhibitors, covalent, and non-covalent inhibitors have been developed with different mechanisms of action (molecular gels, salt-bridging, etc.). MRTX-1133, the fastest progressing G12D non-covalent small molecule inhibitor. It efficiently binds states of KRAS G12D, with more than 500-fold more potency and selectivity than wild KRAS binding, but one of its bigger problems is poor oral

bioavailability. BI-2852, an inhibitor based on the switch SI/II pocket which could block the interaction of GEF, GAP and other effectors and leads to inhibit the signaling downstream and tumor cell proliferation. The mechanism of BI-2852 was different with covalent G12C inhibitors and binding to active G12D 10 times (740 nM vs 7.5 μM) stronger than to KRASwt. The covalent regulatory strategies (YK-8S, R-7), as well as the molecular gel-type inhibitors forming ternary complexes to regulate upstream and downstream effectors and cofactors, were targeted [17, 18]. TH-Z835, suppresses exchange of mantGMPPNP and GPPNP, leading to the MAPK signaling and inhibition of tumor proliferation. The in vivo animal experiment of pancreatic cancer demonstrated that TH-Z835 significantly reduced the tumor size and in synergy with an anti-PD-1 antibody [19].

Other mutation sites, like G12V and G12X had also emerged a lot of treatment options. AFNT-211 (Structural formula undisclosed), an FAS-41BB–promoted TCR-T cell therapy enhance antitumor activity against KRAS G12V-expressing solid tumours. The FAS-41BB switch receptor could promote the anti-cancer response to kill FASL-expressing cancer cells [20]. RMC-7977 (**Figure 2f**), an inhibitor with broad-spectrum activity for active state 'pan' variants, binding to the RAS domain of KRAS proteins, particularly the G12V KRAS mutant, to form a reversible ternary complex [21].

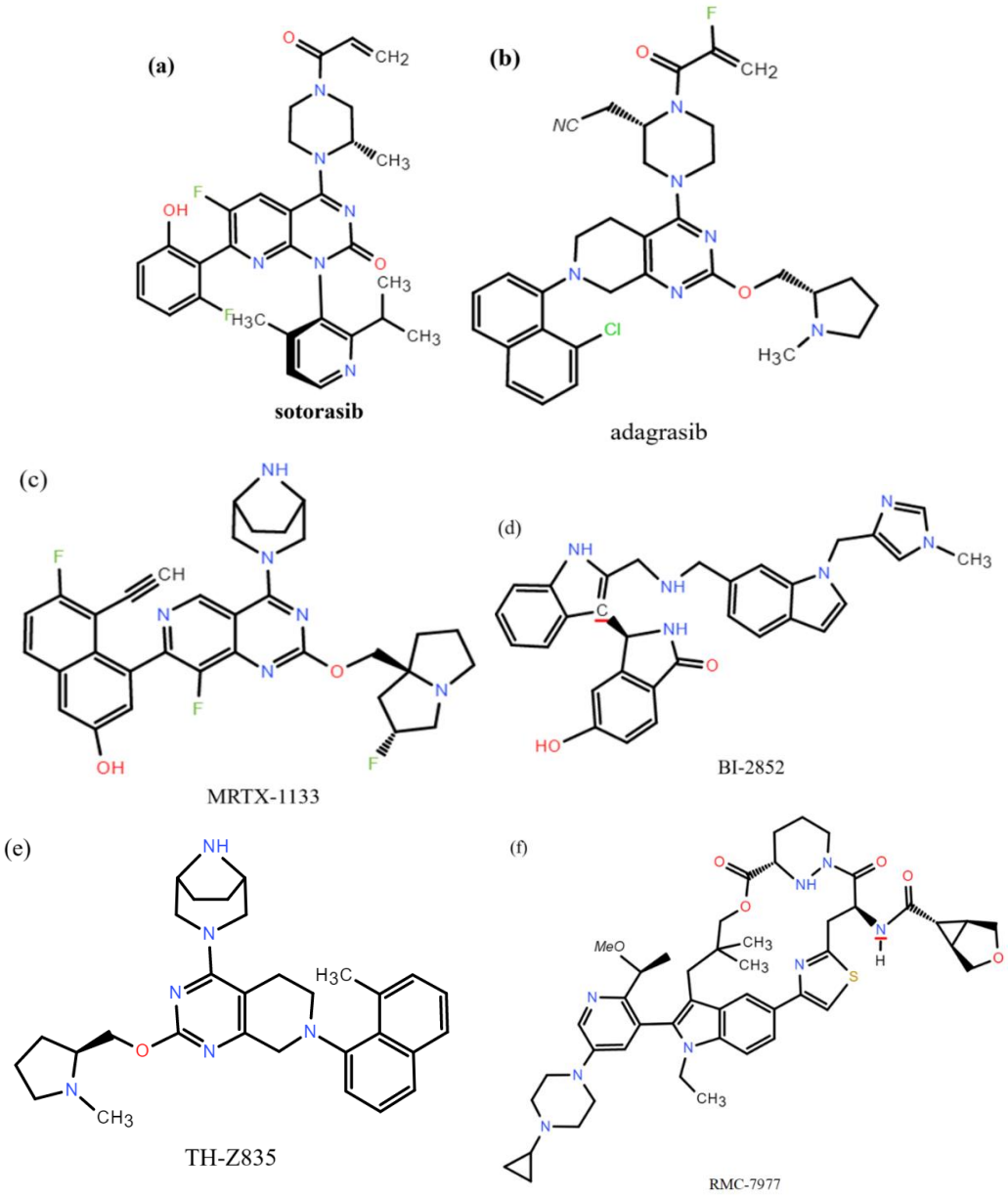
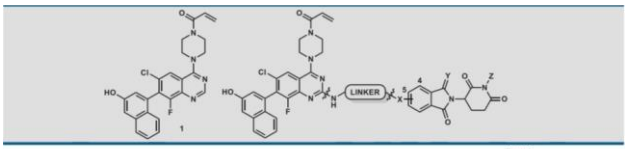


Figure 2. Representative small molecular inhibitors: (a)(b) KRAS G12C inhibitors; (c)(d)(e)(f) G12D inhibitors; (g) G12V inhibitors. [8-23]

Though these drugs mentioned below had showed a brilliant effect to antitumor, drug resistance observed in small molecular inhibitors remains unaddressed[22]. Multiple mechanisms of resistance exist in patients who develop resistance to KRAS inhibitors, and secondary mutations are frequently found in subclonal tumor cell populations. To address this challenge, a lot of work have been done to combat drug form resistance. In principle, there are two main types of drug resistance. Type I comes to the acquired resistance developed via the medication process, since the resistance may be attibuted to KRAS alterations including high-level KRAS amplification and secondary KRAS mutations[23]. Type II comes to the primary resistance, where the patients seem to naturally insensible to the drug. Take G12C inhibitors as an example, the primary resistance comes to 36% of patients experienced on sotorasib therapy published in 2023[24]. From the perspective of genetic mechanisms, Dr. O'Reilly pointed that RAS-MAPK signalling was reestablished for almost every patient with resistance mutations, which indicate the addiction among cancers to RAS signalling. In addition, new drugs targeting multiple secondary alterations, incorporate outside the switch II pocket, change the order of treatment and develop innovative treatment strategies should take more concentrations [12]. To address drug resistance scientists had pay much effort, the following PROTACs is one of them.

3. PROTACs

Proteolysis targeting chimera is also a potential drug design therapy which using a bifunctional chemical linker recruit the E3 ligase leading to the degradation by 16S proteasome. Compared to small molecule inhibitors, the PROTACs have enhanced and long-lasting effects [25-27]. Late in 2020, a PROTACs library for KRAS G12C is synthesized (Figure 3.). XY-4−88 (Figure 4a), a covalent PROTACs using ARS-1620 and thalidomide CRBN-recruiting ligand leading to degradation of an GFP-KRAS G12C fusion protein [28]. LC-2(Figure 4b), the first degrader of endogenous G12C with a MRTX849 as warhead and Thalidomide as the E3 ligase VHL recruiter which induces polyubiquitination and degradation by the 20S proteasome [29]. ASP3082, a potential therapeutic agent for G12D mutation at a phase I clinical trial, which could form a ternary complex by binding to KRAS G12D and E3. This leads to degradation of G12D and inhabitation of ERK phosphorylation of their ASP3082 (structure was not disclosed) [30]. MA06.10, a new potent KRAS G12D-PROTAC molecule, which is not only highly potent in cell proliferation assays, but also exhibits excellent pharmacokinetics [31,32].



	Compound	Linker	x	y	z	IC_{50} (nM)[a]	Protein Level (%)[b]	tPSA (Å^2)[c]
Mixed linker	2		4-NH	O	H	764	100	225
	3		4-NH	O	H	20,330	100	262
	4		4-NH	H_2	H	ND	100	236
	5		4-NH	H_2	H	ND	100	254
PEG linker	6		4-O	O	H	1,118	93	202
	7		4-O	O	H	575	94	211
	8		4-O	O	H	1,606	97	221
	9		4-NH	O	H	104	81	205
Carbon linker	10		4-NH	O	H	ND	99	177
Tertiary amine-containing linker	11		4-NH	O	H	752	75	180
	12		4-NH	O	H	414	76	184
	13 (XY-4-88)		4-NH	O	H	79	68	184
	14		4-NH	O	H	37	83	184
	15		4-NH	O	H	686	90	202
meta-Linkage	16		5-NH	O	H	1,350	96	202
	17		5-NH	O	H	115	65	184

Figure 3. Design of the KRAS G12C Degrader Library [30].

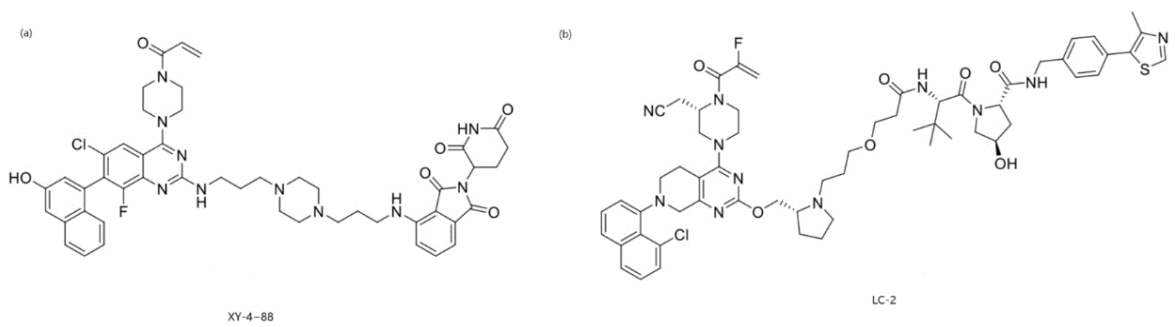


Figure 4. Chemical structures of representative different PROTACs [30-31].

Overall, the selection of warhead and E3 ubiquitinates is indeed important in the selection of PROTACs. For E3 ubiquitinates, here are strategies. One of them is the shape complementarity between the target and the ligase, and then ability of the ligase forming a ternary complex with degradation capacity. However, formation of a ternary complex with high efficiency and maintenance of a tight binding is not necessarily in positive corelation with efficient degradation.

4. Other strategies

As mentioned above, KRAS mutations, like other driver oncogenes, have an impact on the tumour microenvironment as well as altering behaviour of tumour cells. KRAS has a variety of immunomodulatory effects that can be mediated through multiple mechanisms (Figure 5.). It has been found that KRAS oncogenic mutations can promote immune escape by promoting immunosuppressive TME, and this suppression can be mitigated by inhibition of SOS1 or SHP2 - possibly a potential combined strategy [31]. Based on this, a lot of different types of small molecular inhibitors is explored in a number of clinical trials, including CodeBreaK 101, CodeBreaK 100, KRYSTAL-7, KRYSTAL-1, KontRASt-01and GO421447 [33].

The tumour mutational heterogeneity and the specific co-mutations could be the theoretical mechanism of primary resistance. To understand the mechanism of drug resistance may help to develop a new treatment strategy. Therefore, efficient elimination of KRAS mutations may offer an implementory therapy for KRAS inhibition [34]. Lee and coworkers reported that disruption of G12D mutation via CRISPR/Cas9 could slow down the proliferation along with ingenuity of tumour cells [35, 36].

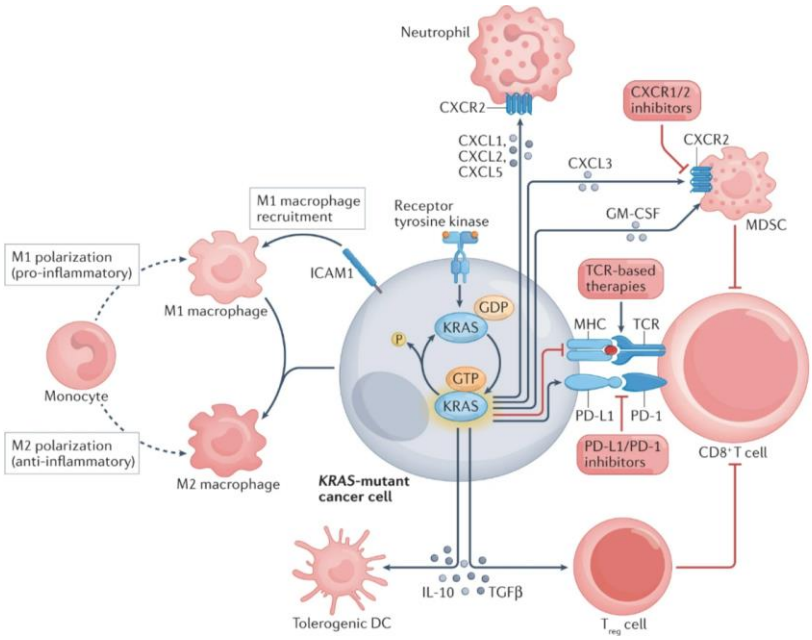


Figure 5. Impact of mutant KRAS [35].

5. Conclusion

This review summarizes a series of strategies targeting some classical types of KRAS mutations protein, including small molecular inhibitors, PROTACs and others. Each approach has its own advantages and disadvantages - small molecule inhibitors are easier to target **KRAS,** but their drug efficacy is uncertain. PROTACs are potent and can mediate ubiquitination degradation but are difficult to bind to proteins due to their high molecular weight. Nevertheless, many scientists are still dedicated to the development of new drugs and the exploration of pharmaceutical strategies. This review lists the current state of affairs and will hopefully inspire those working in the field, or those who wish to join the field.

References

[1] Martinez P et al 2016 *Nat. Commun*. **7** 12158.

[2] Santos E and Nebreda A R 1989 *Faseb J.* **3** 2151-63

[3] Vetter I R and Wittinghofer A 2001 *Science* **294** 1299-304.

[4] Carvalho Dias P et al 2018 *Cancer Res*. **78** 7-14

[5] Hobbs G A, Der C J and Rossman K L 2016 *J. Cell Sci.* **129** 1287-92

[6] Xie X et al 2023 *Signal Transduction and Targeted Therapy* **8** 335

[7] Huang L et al 2021 *Signal Transduction and Targeted Therapy* **6** 386

[8] FDA Approves First KRAS Inhibitor: Sotorasib 2021*Cancer Discovery* **11** Of4.

[9] Hallin J et al 2020 *Cancer Discovery* **10** 54-71

[10] Salem M E et al 2022 *JCO Precis Oncol*. **6** e2100245.

[11] Dong X et al 2024 *Cancer Research* **84** CT119

[12] Singhal A, Li B T and O'Reilly E M 2024 *Nature Medicine* **30** 969-983

[13] Wang X et al 2022 *Journal of Medicinal Chemistry* **65** 3123-3133

[14] Wei D et al 2024 *Clin Cancer Res*. **30** 655-662

[15] Zhou X, Ji Y and Zhou J. 2023 *Molecules* **28** 3615

[16] Nagashima T et al 2022 *European Journal of Cancer* **174** S30

[17] Tran T H et al 2020 *Proceedings of the National Academy of Sciences of the United States of America* **117** 3363-3364

[18] Escher T and Satchell K 2023 *Molecular Therapy* **31** 1904-1919

[19] Mao Z et al 2022 *Cell Discovery* **8** 5

[20] Hoffmann M et al 2023 *Journal of Clinical Oncology* **41** 2543

[21] Holderfield M et al 2024 *Nature* **629** 919–926

[22] Mark A M et al 2021 *New England Journal of Medicine* **384** 2382-2393

[23] Xue J Y et al 2020 *Nature* **577** 421-425

[24] Dy G K et al 2023 *J Clin Oncol*. **41** 3311-3317

[25] Yang F et al 2022 *European Journal of Medicinal Chemistry* **230** 114088

[26] Toure M and Crews C M 2016 *Angew. Chem. Int. Engl*. **55** 1966-1973

[27] Burslem G M et al 2018 *Cell Chem. Biol*. **25** 67-77

[28] Zeng M et al 2020 *Cell Chemical Biology* **27** 19-31

[29] Bond M J et al 2020 *ACS Central Science* **6** 1367-1375

[30] Tolcher A W et al 2023 *Journal of Clinical Oncology* **41** TPS764

[31] Lake D 2016 *Cell Mol. Life Sci*. **73** 4397-4413

[32] Smith K et al 2023 *Molecular Cancer Research* **21** PR09

[33] Punekar S R et al 2022 *Nature Reviews Clinical Oncology* **19** 637-655

[34] Watanabe T et al 2011 *Dis. Colon. Rectum*. **54** 1170-8

[35] Lee W et al 2018 *Scientific Reports* **8** 11879

[36] Kim W et al 2018 *Genome Res*. **28** 374-82