
***Panić L, Tamarut S, Sticker-Jantscheff M, Barkić M, Solter D, Uzelac M, et al. Ribosomal protein S6 gene haploinsufficiency is associated with activation of a p53-dependent checkpoint during gastrulation. *Mol Cell Biol.* 2006;26:8880-91.**

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Nascent ribosome biogenesis is required during cell growth. To gain insight into the importance of this process during mouse oogenesis and embryonic development, we deleted one allele of the ribosomal protein S6 gene in growing oocytes and generated S6-heterozygous embryos. Oogenesis and embryonic development until embryonic day 5.5 (E5.5) were normal. However, inhibition of entry into M phase of the cell cycle and apoptosis became evident post-E5.5 and led to perigestration lethality. Genetic inactivation of p53 bypassed this checkpoint and prolonged development until E12.5, when the embryos died, showing decreased expression of D-type cyclins, diminished fetal liver erythropoiesis, and placental defects. Thus, a p53-dependent checkpoint is activated during gastrulation in response to ribosome insufficiency to prevent improper execution of the developmental program.

Hasenoechl A, Galić T, Ergović G, Maršić N, Skerlev M, Mittendorf J, et al. In vitro activity and in vivo efficacy of icofungipen (PLD-118), a novel oral antifungal agent, against the pathogenic yeast *Candida albicans*. *Antimicrob Agents Chemother.* 2006;50:3011-8.

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Icofungipen (PLD-118) is the representative of a novel class of antifungals, beta amino acids, active against *Candida* species. It has been taken through phase II clinical trials. The compound actively accumulates in yeast, competitively inhibiting isoleucyl-tRNA synthetase and consequently disrupting protein biosynthesis. As a result, in vitro activity can be studied only in chemically defined growth media without free amino

acids that would compete with the uptake of the compound. The MIC of icofungipen was reproducibly measured in a microdilution assay using yeast nitrogen base medium at pH 6 to 7 after 24 h of incubation at 30 to 37 degrees C using an inoculum of 50 to 100 CFU/well. The MICs for 69 *Candida albicans* strains ranged from 4 to 32 microg/ml. This modest in vitro activity contrasts with the strong in vivo efficacy in *C. Albicans* infection. This was demonstrated in a lethal model of *C. albicans* infection in mice and rats in which icofungipen showed dose-dependent protection at oral doses of 10 to 20 mg/kg of body weight per day in mice and 2 to 10 mg/kg/day in rats. The in vivo efficacy was also demonstrated against *C. albicans* isolates with low susceptibility to fluconazole, indicating activity against azole-resistant strains. The efficacy of icofungipen in mice and rats was not influenced by concomitant administration of equimolar amounts of L-isoleucine, which was shown to antagonize its antifungal activity in vitro. Icofungipen shows nearly complete oral bioavailability in a variety of species, and its in vivo efficacy indicates its potential for the oral treatment of yeast infections.

Rosandić M, Paar V, Basar I, Glunčić M, Pavin N, Pilas I. CENP-B box and pJalpha sequence distribution in human alpha satellite higher-order repeats (HOR). *Chromosome Res.* 2006;14:735-53.

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Using their Key String Algorithm (KSA) to analyze Build 35.1 assembly, the authors determined consensus alpha satellite higher-order repeats (HOR) and consensus distributions of CENP-B box and pJalpha motif in human chromosomes 1, 4, 5, 7, 8, 10, 11, 17, 19, and X. They determined new supra-chromosomal family (SF) assignments: SF5 for 13mer (2211 bp), SF5 for 13mer (2214 bp), SF2 for 11mer (1869 bp), SF1 for 18mer (3058 bp), SF3 for 12mer (2047 bp), SF3 for 14mer (2379 bp), and SF5 for 17mer (2896 bp) in chromosomes 4, 5, 8, 10, 11, 17, and 19, respectively. In chromosome 5 they identified SF5 13mer without any CENP-B box and pJalpha motif, highly homologous (96%) to 13mer in chromosome 19. Addi-

tionally, in chromosome 19 the authors identified new SF5 17mer with one CENP-B box and pJalpha motif, aligned to 13mer by deleting four monomers. In chromosome 11 they identified SF3 12mer, homologous to 12mer in chromosome X. In chromosome 10 they identified new SF1 18mer with eight CENP-B boxes in every other monomer (except one). In chromosome 4 they identified new SF5 13mer with CENP-B box in three consecutive monomers. The authors found four exceptions to the rule that CENP-B box belongs to type B and pJalpha motif to type A monomers.

Mahmutefendić H, Blagojević G, Kučić N, Lučin P. Constitutive internalization of murine MHC class I molecules. J Cell Physiol. 2006;210:445-55.

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In this study the authors compared spontaneous internalization of murine major histocompatibility complex (MHC) class I molecules (K(d), D(d), full L(d), and empty L(d)) after depletion of their egress to the cell surface (Cycloheximide [CHX], brefeldin A [BFA]) and internalization after external binding of monoclonal antibody (mAb). MHC class I alleles differ regarding their cell surface stability, kinetics, and in the way of internalization and degradation. K(d) and D(d) molecules are more stable at the cell surface than L(d) molecules and, thus, constitutively internalized more slowly. Although the binding of mAbs to cell surface MHC class I molecules results in faster internalization than depletion of their egress, it is still slow and, thereby, can serve as a model for tracking of MHC class I endocytosis. Internalization of fully conformed MHC class I molecules (K(d), D(d), and L(d)) was neither inhibited by chlorpromazine (CP) (inhibitor of clathrin endocytosis), nor with filipin (inhibitor of lipid raft dependent endocytosis), indicating that fully conformed MHC class I molecules are internalized via the bulk pathway. In contrast, internalization of empty L(d) molecules was inhibited by filipin, indicating that non-conformed MHC class I molecules require intact cholesterol-rich membrane microdomains for their constitutive internalization. Thus, conformed and non-conformed MHC class I molecules use different endocytic pathways for constitutive internalization.

***Majhen D, Gabrilovac J, Eloit M, Richardson J, Ambriović-Ristov A. Disulfide bond formation in NGR fiber-modified adenovirus is essential for retargeting to**

aminopeptidase N. Biochem Biophys Res Commun. 2006;348:278-87.

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The peptide motif NGR (asparagine-glycine-arginine) is known to bind to aminopeptidase N (APN). The authors have constructed five adenoviruses (Ads) bearing NGR in the HI loop of the adenoviral fiber protein. They compared the targeting properties of the NGR peptide within different amino acid environments and showed that their cellular receptor(s) were not identical. Ads containing NGR within potentially cyclic sequences flanked by cysteines retargeted viruses mainly to APN, while Ads containing NGR within linear sequences not containing cysteines retargeted Ads mainly to alpha(v)beta(3) integrin, albeit with a lower affinity. Finally, the authors show evidence that disulfide bond formation within an Ad bearing the CDCNGRCFC sequence is essential for retargeting to APN, suggesting that this sequence does indeed assume a cyclic structure which facilitates NGR binding to APN. Therefore, this study underscores the importance of cysteine residues flanking targeting peptides for not only affinity but also specificity of the retargeted Ad.

Lučić Vrdoljak A, Čalić M, Radić B, Berend S, Jun D, Kuća K, et al. Pretreatment with pyridinium oximes improves antidotal therapy against tabun poisoning. Toxicology. 2006;228:41-50.

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Oximes K033 [1,4-bis(2-hydroxyiminomethylpyridinium)butane dibromide] and K048 [1-(4-hydroxyiminomethylpyridinium)-4-(4-carbamoylpyridinium)butane dibromide] were tested as pretreatment drugs in tabun-poisoned mice followed by treatment with atropine plus K033, K048, K027 [1-(4-hydroxyiminomethylpyridinium)-3-(4-carbamoylpyridinium)propane dibromide], TMB-4 [1,3-bis(4-hydroxyiminomethylpyridinium)propane dibromide] and HI-6 [(1-(2-hydroxyiminomethylpyridinium)-3-(4-carbamoylpyridinium)-2-oxapropane dichloride)]. Oxime doses of 25% or 5% of its LD₅₀ were used for pretreatment 15 min before tabun-poisoning and for treatment 1 min after tabun administration to mice. The best therapeutic effect was obtained when oxime K048 (25% of its LD₅₀) was used in both pretreatment and treatment with atropine. This regimen insured survival of all tested animals after the application of 10 LD₅₀ of tabun. In addition, since butyrylcho-

linesterase (BChE; EC 3.1.1.8) is considered an endogenous bioscavenger of anticholinesterase compounds and its interactions with oximes could be masked by AChE interactions, the authors evaluated kinetic parameters for interactions of tested oximes with native and tabun-inhibited human plasma BChE and compared them with results obtained previously for human erythrocyte acetylcholinesterase (AChE; EC 3.1.1.7). Progressive inhibition of BChE by tabun was slightly faster than that of AChE. The reactivation of tabun-inhibited BChE by oximes was very slow, and BChE binding affinity for oximes was lower than AChE's. Therefore, BChE could scavenge tabun prior to AChE inhibition, but fast oxime-assisted reactivation of tabun-inhibited AChE or protection of AChE by oxime against inhibition with tabun would not be obstructed by interaction between BChE and oximes.

Ćuković-Čavka S, Vermeire S, Hrstić I, Claessens G, Kolaček S, Jakić-Razumović J, et al. NOD2/CARD15 mutations in Croatian patients with Crohn's disease: prevalence and genotype-phenotype relationship. Eur J Gastroenterol Hepatol. 2006;18:895-9.

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Crohn's disease (CD) is a chronic inflammatory disorder of the gastrointestinal tract with variations in localization and behaviour. Mutations in the NOD2/CARD15 gene on chromosome 16q have been implicated in the pathogenesis of the disease and three main sequence variants, all single nucleotide polymorphisms (SNPs), have been identified in North American and European populations. As no data exist in the Croatian population, the authors consecutively collected a cohort of 136 CD patients and 91 healthy controls to determine the prevalence of NOD2/CARD15 mutations and their association with phenotypic expression of the disease. All patients and controls were genotyped for Arg702Trp (Hugot SNP8), Gly908Arg (Hugot SNP12), and Leu1007fsinsC (Hugot SNP13) and allele frequencies were compared between the Crohn's patients and controls. The correlation of NOD2/CARD15 genotypes with the phenotypic expression of Crohn's disease was further assessed by logistic regression analysis. NOD2/CARD15 variants were found in 38/136 CD patients (27.9%) compared to 10/91 (10.9%) healthy controls ($p=0.0022$). Allele frequencies in patients with CD were 13.97%, 4.4% and 11.76%, respectively, for SNP8, 12 and 13, compared to 5.49%, 1.12%

and 4.40% in controls ($p=0.041$, $p=0.162$, $p=0.055$). Six CD patients carried double mutations and, remarkably, the authors identified two homozygous mutants amongst the healthy control group. Surgery over the course of the disease and a younger age at onset of the disease were significantly more frequent in patients who were carriers of NOD2/CARD15 mutations. In conclusion, this report on NOD2/CARD15 mutations in Croatian patients with CD demonstrates that this gene is also implicated in susceptibility to CD in the Croatian population. Phenotypic association showed a younger age at diagnosis and a higher need for surgery in patients carrying NOD2/CARD15 mutations. However, the prevalence is somewhat lower compared to other reports, likely due to a more prominent colonic inflammation.

Pivac N, Kozarić-Kovačić D, Mustapić M, Dezeljin M, Borovečki A, Grubišić-Ilić M, et al. Platelet serotonin in combat related posttraumatic stress disorder with psychotic symptoms. J Affect Disord. 2006;93:223-7.

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Combat-related posttraumatic stress disorder (PTSD) is severe form of PTSD, frequently associated with psychotic symptoms. Platelet serotonin (5-hydroxytryptamine, 5-HT) was used as a peripheral 5-HT marker to identify particular symptoms in PTSD. Platelet 5-HT was determined fluorimetrically in 67 war veterans with combat related PTSD, 36 combat exposed veterans who did not develop PTSD, 35 veterans with PTSD complicated with psychotic features. PTSD diagnosis of current and chronic PTSD, and clinical symptoms of PTSD and psychoses were assessed according to DSM-IV criteria, using the Clinician Administered PTSD Scale, and Positive and Negative Syndrome Scale (PANSS). Platelet 5-HT concentration was significantly higher in veterans with psychotic PTSD than in veterans with non-psychotic PTSD, veterans without PTSD, or in control subjects. Platelet 5-HT was significantly positively correlated with the positive symptoms in PANSS subscale, and with the symptoms of delusions within PANSS positive subscale. Since the delusions are the core psychotic symptoms occurring in psychotic PTSD patients described in this study, the result of the increased platelet 5-HT concentration, associated with delusions, indicate that platelet 5-HT might be used as a trait marker of psychotic symptoms in PTSD, but not as a state marker for PTSD.