Šantak G, Šantak M, Forčić D. Native human IFN-alpha is a more potent suppressor of HDF response to profibrotic stimuli than recombinant human IFN-alpha. J Interferon Cytokine Res. 2007;27:481-90.

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Interferon-alpha(IFN-alpha) inhibits fibroblast proliferation, differentiation into myofibroblasts, and extracellular matrix synthesis, which are key events during both normal wound repair and fibrotic lesion formation. Unlike recombinant human IFN-alpha (rHuIFN-alpha), a native human IFN-alpha (nHuIFN-alpha) consists of several IFN-alpha subtypes and traces of other cytokines produced by the Sendai virus-stimulated human leukocytes. This study compares the antifibrotic effect of nHuIFN-alpha and rHuIFN-alpha in normal human dermal fibroblasts (HDFs). Treatment of HDF culture with nHuIFNA-alpha markedly affects HDF viability, whereas different rHuIFN-alpha subtypes show various effects. Two of twelve rHuIFN-alpha subtypes (IFN-alpha B2 and IFN-alpha K) significantly reduce cell viability of HDFs compared with nontreated HDFs. However, nHuIFN-alpha significantly reduces HDF cell viability in comparison to both nontreated cells and cells treated with rHuIFN-alpha. The 50% inhibitory concentration (IC_{50}) varied 10-fold between nHuIFN-alpha and rHuIFN-alpha (1,103 IU/mL and 10,762 IU/mL, respectively). The impact on procollagen type I mRNA synthesis level is comparable at low doses of IFN (100 and 500 IU/mL), whereas at the dose of 1,000 IU/mL, nHuIFN-alpha shows higher repression of collagen type I gene than does rHuIFN-alpha. Both, nHuIFN-alpha and rHuIFN-alpha antagonize the effect of exogenous transforming growth factor-beta (TGF-beta) and interleukin-4 (IL-4) as measured by the alpha-smooth muscle actin (alpha-SMA) and procollagen type I mRNA level, but the effect of nHuIFN-alpha is more pronounced. This study suggests that nHuIFN-alpha is a more potent suppressor of the HDF response to profibrotic stimuli than rHuIFN-alpha, probably because of the synergism between different IFN-alpha subtypes and antifibrotic cytokines and factors.

Ivančev V, Palada I, Valić Z, Obad A, Baković D, Dietz NM, et al. Cerebrovascular reactivity to hypercapnia is unimpaired in breath-hold divers. J Physiol. 2007;582(Pt 2):723-30.

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Hypercapnic cerebrovascular reactivity is decreased in obstructive sleep apnoea and congestive heart disease perhaps as a result of repeated apnoeas. To test the hypothesis that repeated apnoeas blunt cerebrovascular reactivity to hypercapnia, the authors studied breath hold divers and determined cerebrovascular reactivity by measuring changes in middle cerebral artery velocity (MCAV, cm s^{-1}) per mmHg change in end-tidal partial pressure of CO_2 in response to two hyperoxic hypercapnia rebreathing manoeuvres (modified Read protocol) in elite breath-hold divers (BHD, n=7) and non-divers (ND, n=7). In addition, ventilation and central (beat-to-beat stroke volume measurement with Modelflow technique) haemodynamics were determined. Ventilatory responses to hypercapnia were blunted in BHD versus ND largely due to lower breathing frequency. Cerebrovascular reactivity did not differ between groups (p=0.90) and the same was found for cerebral vascular resistance and MCAV recovery to baseline after termination of the CO_2 challenge. Cardiovascular parameters were not changed significantly during rebreathing in either group, except for a small increase in mean arterial pressure for both groups. These findings indicate that the regulation of the cerebral circulation in response to hypercapnia is intact in elite breath-hold divers, potentially as a protective mechanism against the chronic intermittent cerebral hypoxia and/or hypercapnia that occurs during breath-hold diving. These data also suggest that factors other than repeated apnoeas contribute to the blunting of cerebrovascular reactivity in conditions like sleep apnoea.

Debeljak Z, Škrbo A, Jasprica I, Mornar A, Plečko V, Banjanac M, et al. QSAR study of antimicrobial activity

of some 3-nitrocoumarins and related compounds. J Chem Inf Model. 2007;47:918-26.

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A new class of antimicrobial agents, 3-nitrocoumarins and related compounds, has been chosen as a subject of the present study. In order to explore their activity and molecular properties that determine their antimicrobial effects, QSAR models have been proposed. Most of the 64 descriptors used for the development were extracted from semiempirical and density functional theory (DFT) founded calculations. For this study literature data containing results of microbiological activity screening of 33 coumarin derivatives against selected clinical isolates of C. albicans (CA) and S. aureus (SA) have been selected. Multivariate predictive models based on random forests (RF) and two hybrid classification approaches, genetic algorithms (GA) associated with either support vector machines (SVM) or k nearest neighbor (kNN), have been used for establishment of QSARs. An applied feature selection approach enabled two-dimensional linear separation of active and inactive compounds, which was a necessary tool for rational candidate design and descriptor relevance interpretation. Candidate molecules were checked by cross-validated models, and selected derivatives have been synthesized. Their antimicrobial activities were compared to antimicrobial activities of the representative derivatives from the original set in terms of minimal inhibitory concentration (MIC) against chosen SA and CA ATCC strains. High ranking of descriptors consistent with the degree of hydrolytic instability of selected compounds is common to models of antimicrobial activity against both microorganisms. However, descriptor ranking indicates different antimicrobial mechanisms of action of chosen coumarin derivatives against selected microbial species.

Relja M, Poole AC, Schoenen J, Pascual J, Lei X, Thompson C; European BoNTA Headache Study Group. A multicentre, double-blind, randomized, placebo-controlled, parallel group study of multiple treatments of botulinum toxin type A (BoNTA) for the prophylaxis of episodic migraine headaches. Cephalalgia. 2007;27:492-503.

Department of Neurology, Zagreb University School of Medicine, Zagreb, Croatia

The aim of this study was to evaluate the safety and efficacy of botulinum toxin type A (BoNTA; BOTOX) for prophylaxis of episodic migraine. In this double-blind, placebo-controlled study, patients were randomized to 225, 150 or 75 U of BoNTA or placebo after a 30-day placebo run-in for three 90-day treatment cycles. The primary efficacy end-point was the mean reduction from baseline in the frequency of migraine episodes at day 180 in the placebo non-responder stratum. All groups (N=495) improved, with no significant differences. At day 180, the frequency of migraine episodes was reduced from baseline means of 4.3, 4.7, 4.7 and 4.4 by 1.6, 1.7, 1.5 and 1.4 for BoNTA 225 U, 150 U and 75 U and placebo, respectively. The primary end-point was not met. Treatment-related adverse events were transient and mild to moderate. BoNTA treatment was safe and well tolerated but did not result in significantly greater improvement than placebo in this study. Several factors may have confounded the results.

Tomić S, Bertosa B, Wang T, Wade RC. COMBINE analysis of the specificity of binding of Ras proteins to their effectors. Proteins. 2007;67:435-47.

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Understanding the interaction of Ras with other proteins is of importance not only for studying signalling mechanisms but also, because of their medical relevance as targets, for anticancer therapy. To study their selectivity and specificity, which are essential to their signal transfer function, the authors performed COMparative BINding Energy (COMBINE) analysis for 122 different wild-type and mutant complexes between the Ras proteins, Ras and Rap, and their effectors, Raf and RalGDS. The COMBINE models highlighted the amino acid residues responsible for subtle differences in binding of the same effector to the two different Ras proteins, as well as more significant differences in the binding of the two different effectors (RalGDS and Raf) to Ras. The study revealed that E37, D38, and D57 in Ras are nonspecific hot spots at its effector interface, important for stabilization of both the RalGDS-Ras and Raf-Ras complexes. The electrostatic interaction between a GTP analogue and the effector, either Raf or RalGDS, also stabilizes these complexes. The Raf-Ras complexes are specifically stabilized by S39, Y40, and D54, and RalGDS-Ras complexes by E31 and D33. Binding of a small molecule in the vicinity of one of these groups of amino acid residues could increase discrimination between the Raf-Ras and RalGDS-Ras complexes. Despite the different size of the RalGDS-Ras and Raf-Ras complexes, the authors succeeded in building COMBINE models for one type of complex that were also predictive for the other type of protein complex. Further, using system-specific models

trained with only five complexes selected according to the results of principal component analysis, the authors were able to predict binding affinities for the other mutants of the particular Ras-effector complex. As the COMBINE analysis method is able to explicitly reveal the amino acid residues that have most influence on binding affinity, it is a valuable aid for protein design.

Mitrović Z, Aurer I, Radman I, Ajduković R, Sertić J, Labar B. FCgammaRIIIA and FCgammaRIIA polymorphisms are not associated with response to rituximab and CHOP in patients with diffuse large B-cell lymphoma. Haematologica. 2007;92:998-9.

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The authors investigated the association of FcgammaRII-Iaa and FcgRIIa polymorphisms and response to R-CHOP in 58 patients with diffuse large B-cell lymphoma (DLBCL). FcgammaRIIIa and FcgRIIa polymorphisms did not influence response, event-free or overall survival. These results suggest that ADCC via FcgammaRIIIa and FcgammaRIIa may not be the major mechanism of activity of the R-CHOP combination in DLBCL.

Halassy B, Vdović V, Habjanec L, Balija ML, Gebauer B, Sabioncello A, et al. Effectiveness of novel PGM-containing incomplete Seppic adjuvants in rabbits. Vaccine. 2007;25:3475-81.

Research and Development Department, Institute of Immunology, Inc., Zagreb, Croatia

Peptidoglycan monomer (PGM) is adjuvant active molecule in experimental mice, although its adjuvanticity is much lower in comparison to potent adjuvants. The novel adjuvant formulations were developed by incorporation of PGM into Montanide ISA 206 and Montanide ISA 720 adjuvants, with the aim to enhance its adjuvanticity by protecting it from the fast degradation and metabolic clearance. Adjuvanticity of the novel adjuvant formulations was tested in rabbits for induction of protein-specific antibodies. Both novel adjuvants ISA206(PGM) and ISA720(PGM) were significantly stronger than Montanide adjuvants themselves, and also significantly more potent than Complete Freund Adjuvant. Montanide ISA 720 was shown as much better carrier of PGM, since the novel ISA720(PGM) adjuvant was significantly stronger adjuvant than the ISA206(PGM).

Detel D, Peršić M, Varljen J. Serum and intestinal dipeptidyl peptidase IV (DPP IV/CD26) activity in children with celiac disease. J Pediatr Gastroenterol Nutr. 2007;45:65-70.

Department of Chemistry and Biochemistry, Rijeka University School of Medicine, Rijeka, Croatia

Dipeptidyl peptidase IV (DPP IV/CD26) is involved in the degradation of proline-rich proteins such as gliadin and in modulation of the immune response. The aim of this study was to examine the possible causal connection between DPP IV enzyme activities and celiac disease (CD) in children. Intestinal mucosal biopsy specimens were obtained from 97 patients. The patients were divided into 3 groups: patients with active CD (n=38), patients with malabsorption syndrome (MS) of other causes (n=37), and control patients (n=22). In addition, blood samples were collected from 48 patients with active CD and 50 control patients without gastrointestinal diseases. DPP IV enzyme activity was measured in the intestinal mucosal biopsy specimens and in the serum samples. DPP IV activity in the small intestine correlated inversely with the grade of mucosal damage in the CD ($r=-0.92$, $p<0.001$) and MS groups ($r=-0.90$, $p<0.001$). Intestinal DPP IV activity was statistically significantly lower in the CD and MS groups than in the control group ($p< 0.001$). By contrast, serum DPP IV activity was not significantly different between the CD and control groups. These results suggest that the decrease in intestinal DPP IV activity is not specific to CD because it correlates with the level of mucosal damage in both patients with CD and those with MS. In addition, it seems that serum DPP IV activity cannot be used as a specific noninvasive diagnostic or prognostic marker of CD.

Vukelić Z, Kalanj-Bognar S, Froesch M, Bindila L, Radić B, Allen M, et al. Human gliosarcoma-associated ganglioside composition is complex and distinctive as evidenced by high-performance mass spectrometric determination and structural characterization. Glycobiology. 2007;17:504-15.

Department of Chemistry and Biochemistry, Zagreb University School of Medicine, Zagreb, Croatia

The authors describe the first systematic ganglioside (GG) composition characterization in human gliosarcoma versus normal brain tissue using our recently developed mass spectrometry (MS) methods, based on nano-electrospray (nano-ESI), Fourier-transform ion cyclotron resonance (FT-ICR), and chip nano-ESI quadrupole time-of-flight

(QTOF), complemented by thin-layer chromatographic (TLC) analysis and quantification. Combined MS enabled detection and structural assignment of 73 distinct GG species: many more than reported so far for investigated gliomas. Apart from the 7.4-times lower total GG content, gliosarcoma contained all major brain-associated species, however, in very altered proportions, exhibiting a highly distinctive pattern: GD3 (48.9%)>GD1a/nLD1>GD2/GT3>GM3>GT1b>GM2>GM1a/GM1b/nLM1>LM1>GD1b>GQ1b. MS also revealed abundant O-Ac-GD3; its sequencing provided structural evidence to postulate a novel O-Ac-GD3 isomer O-acetylated at the inner Neu5Ac-residue, previously not structurally confirmed. The high sensitivity and mass accuracy permitted the assignment of unusual minor species: GM4, Hex-HexNAc-nLM1, Gal-GD1, Fuc-GT1, GalNAc-GT1, O-Ac-GM3, di- O-Ac-GD3O-Ac-GD3, and O-Ac-GT3, not previously reported as glioma-associated. The gliosarcoma-expressed GA2 might represent a marker distinguishing astrocytic from oligodendroglial tumors. This is, to the authors' knowledge, so far the most complete GG composition characterization of certain glioma, which demonstrates that their MS-based approach could provide essential structural information relevant to glycosphingolipid role(s) in brain tumor biology, differential diagnosis/prognosis and novel treatment concepts.

Lazić R, Gabrić N. Verteporfin therapy and intravitreal bevacizumab combined and alone in choroidal neovascularization due to age-related macular degeneration. Ophthalmology. 2007;114:1179-85.

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The aim was to evaluate the efficacy and safety of photodynamic therapy (PDT) with verteporfin combined with intravitreal bevacizumab in choroidal neovascularization (CNV) owing to age-related macular degeneration (AMD) in comparison with individual monotherapies used as controls in a randomized controlled pilot clinical trial. The patients included males or females, aged ≥ 50 years, with minimally classic or occult CNV owing to AMD in at least 1 eye that had never been treated previously. One hundred sixty-five eyes in 165 subjects (53 males, 112 females) aged between 60 and 87 years (mean [standard deviation]: 75.7 [6.0] years) were randomly assigned to receive either a single PDT session with verteporfin (PDT group; n=55), or a single administration of intravitreal bevacizumab (1.25 mg; BEV group; n=55), or their combination (COMB group; n=55). In the COMB group, bevacizumab was administered within 1 hour of PDT. Subjects were followed up at 1 and 3 months after treatment. Ophthalmic evaluations including optical coherence tomography, fluorescein angiography, and visual acuity (VA) and central foveal thickness (CFT) measurements were performed at each visit. Changes from baseline in best-corrected VA and CFT were measured at 1- and 3-month follow-up visits. One hundred fifty-six subjects (54 BEV, 50 PDT, and 52 COMB) completed the study. At the 3-month follow-up, significant improvements in best-corrected VA were observed in the BEV and COMB groups (0.079 and 0.223 logarithm of the minimum angle of resolution [logMAR], respectively; p<0.0001 for both). In the PDT group, a slight worsening was noted. Significant reductions of CFT were observed in the 3 groups (-34.0 microm [BEV], -59.6 microm [COMB], and -50.5 microm [PDT]; p<0.0001 for all). At the 1-month follow-up, 46 subjects (16 BEV, 29 COMB, and 1 PDT) had an improvement >0.2 logMAR in best-corrected VA; at 3-month follow-up, this improvement persisted in 23 subjects (1 BEV, 22 COMB, and 0 PDT). In conclusion, nificant improvements in best-corrected VA after 1 month and their maintenance over a 3-month period were observed after verteporfin PDT combined with intravitreal bevacizumab. These results should be confirmed in larger and long-term prospective randomized trials.