Chapter 2

Chemotherapy of Adenovirus Infections

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Additional information is available at the end of the chapter

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Abstract

Adenoviruses occupy a substantial place as causative agents of seasonal respiratory infections, the most characteristic and severe being epidemic keratoconjunctivitis (EKC). Moreover, adenovirus infections are very characteristic with their severe course in persons with impaired immune system. The absence of specific anti-adenovirals is the major problem, and the development of compounds effective against adenoviruses is a principal task. This chapter embraces the results of studies on search for antivirals with anti-adenovirus activity, nucleoside/nucleotide analogues and nonnucleoside compounds. Ganciclovir and cidofovir demonstrated effects against adenovirus serotypes *in vitro* and in animal ocular infection models. Cidofovir applied alone or in combination with cyclosporine manifested therapeutic effects on patients with EKC in a controlled clinical study. We characterized abitylguanide as anti-adenovirus agent in broad-scale investigations, including cell culture experiments, and in two double-blind trials with very beneficial results.

Keywords: human adenoviruses, antivirals, nucleosides/nucleotides, nonnucleosides, mode of action, clinical trials

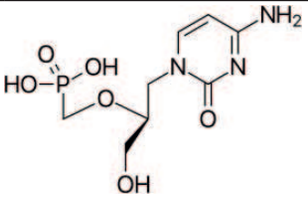
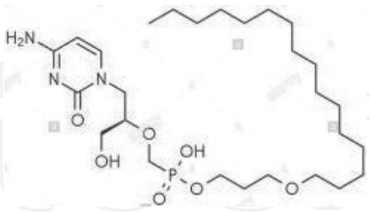
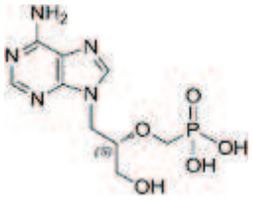
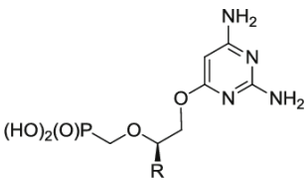
1. Role of adenoviruses in human pathology

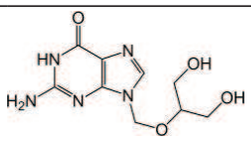
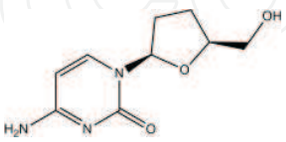
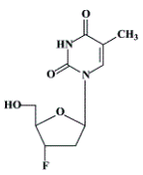
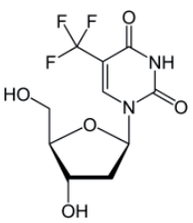
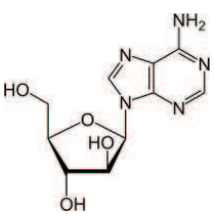
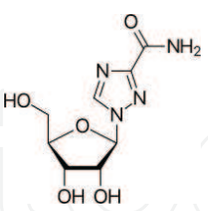
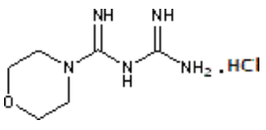
Adenoviruses (AdVs) [1] occupy a significant place in the human pathology [2]. It was established that 30–70% of the human population in Europe and North America shows a seroprevalence to AdVs (cit. in [3]). These viruses are causative agents of wide range of human diseases, showing varying tissue tropism. They are generally middle and self-limiting. Among them, large place is occupied by acute respiratory tract diseases, especially in children [4]. Conjunctivitis is very often registered in these infections. More severe course manifests viral

gastroenteritis, especially in infants, as well as hemorrhagic cystitis, and in rare cases, hepatitis, myocarditis, meningoencephalitis or nephritis [5, 6]. AdVs are characteristic with their severe course in persons with hereditary decreased or impaired immune system: (1) hereditary immunodeficiency; (2) in persons transplant recipients treated with immunosuppressive agents; (3) AIDS. In such patients, the abovementioned clinical manifestations are particularly prone to disseminated disease frequently show a fatal outcome, in children mortality rate attains 83% [7–9]. EKC is another serious and very frequent AdV induced disease, extremely often with social importance [10–14].

The major problem of AdVs infections is the absence of chemotherapeutic agents not only for the clinical practice, but even the absence at strong anti-adenovirals in experimental research. This is pointed in all manuals of virology considering AdVs and AdV-induced infections, in the review articles and even in all encyclopedic sources. Evidently, development of an effective antiviral treatment is a principal task.

This chapter presents a concentrated view on the investigations of experimental chemotherapy of AdV infections and results of their clinical application (**Table 1**).

Compound name	Chemical structure	Target	Way of administration and clinical trial
Cidofovir		AdV DNA polymerase	*Local application in EKC patients* *Oral application in immunocompromised patients* VISTID Controlled clinical pilot study of combination with cyclosporine
Brincyclovir		AdV DNA polymerase	*Oral application* CHIMERIX Phase II clinical trial
(S)-HPMPA		AdV DNA chain elongation	
(S)-HPMPO-DAPy		AdV DNA polymerase	

Compound name	Chemical structure	Target	Way of administration and clinical trial
Ganciclovir		AdV DNA synthesis, inhibition of late genes expression	*Local application* ZIRGAN Three double-blind trials: two in US and one in Germany (40 placebo and 40 ZIRGAN treated each) GCV in combination with the microbicide povidon-iodine
Zalcitabine			
Alovudine		AdV DNA polymerase	
Trifluridine		DNA synthesis	*Local application*
Vidarabine		DNA synthesis	
Ribavirin		• Inhibition of inosin-5-MP dehydrogenese (decreased GTP pools) • Viral polymerases inhibition (RNA capping activity of viral transcripts) • Lethal mutagenesis of viral RNA genomes ("error catastrophe")	*General application in immunocompromized patients*
Abitylguanide-HCl		Ligand of AdV capsid proteins	*Local application in EKC patients* ADENOSTATIN COLLYRIUM Two placebo-controlled randomised trials on 349 EKC patients (151 and 198, respectively) carried out in 1973/74; three placebo-controlled trials in latest 80th years

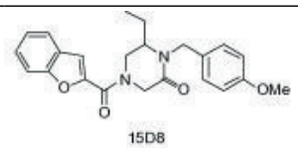
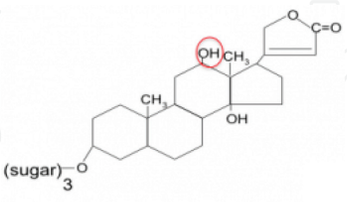
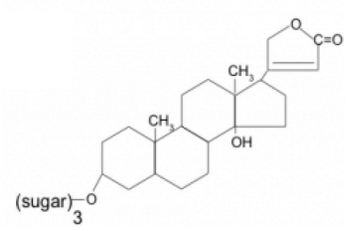
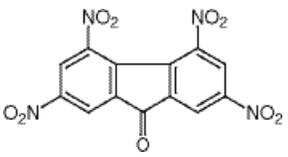
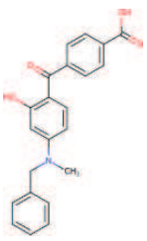
Compound name	Chemical structure	Target	Way of administration and clinical trial
15D8	 15D8	Ligand of precursors of terminal protein pTP, AdV DNA polymerase and DNA-binding protein	
Digoxin and Digitoxin	 	Alter RNA splicing EIA activity gene early in AdV infection (late proteins E4 and F6 and the major late capsid hexon protein are compromised)	*General application(?)*
2,4,5,7-Tetranitro-9-fluorenone		Inhibits AdV cysteine protease (indispensable for the production of infectious AdV virions)	
4-{4-[Benzyl(methyl) amino]-2-hydroxybenzoyl} benzoic acid			

Table 1. Compounds manifesting activity against human adenoviruses.

2. Nucleoside/nucleotide analogues

2.1. Cidofovir [(S)-HPMPC; (S)-1-(3-hydroxy-2-phosphonomethoxypropyl)cytosine; VISTID]

The antiviral effect of cidofovir is based on its transformation in the infected cells in di- and triphosphate and such way becoming an alternative substrate of the AdV DNA polymerase possessing higher affinity compared to cellular DNA polymerases (I, II and III) [15]. Sequence changes in a conserved region of the AdV DNA polymerase were established at cidofovir-resistant AdV mutants [16].

Cidofovir IC_{50} values in cell culture testing versus broad spectrum of AdVs are within 0.8–17 μg/ml [17]. In in vivo testing in ocular infection with AdV (type C) of New Zealand rabbits and cotton rats the compound treatment is efficacious when administered as 0.5–1% eye drops

[18–20]. Romanowski and Gordon [21] found efficacy of topical 0.5% cidofovir on several human adenoviruses (AdV1, AdV5 and AdV6) in the New Zealand rabbit ocular model. AdV type B and type C-induced pneumonia registered in mice and in cotton rats [22] could be used for in vivo treatment with antivirals.

2.2. Brincyclovir (BCV; hexadecyloxypropyl-cidofovir; CMX001)

This compound is a lipidic conjugate of cidofovir. It prevents AdV induced mortality in a permissive, immunosuppressed animal model [23].

2.3. (S)-HPMPA [(S)-9-(3-hydroxy-2-phosphonomethoxypropyl)adenine

Its anti-AdV effect has the same mechanism as cidofovir—inhibition of the AdV DNA chain elongation [24].

2.4. USC-187 (alkyl tyrosinamide-ester prodrug of HPMPA)

This compound proved active against multiple AdV serotypes in vitro and was effective versus AdV-C6 in hamsters immunosuppressed by cyclophosphamide. Administered orally USC-187 prevented or significantly decreased mortality, virus titers and liver pathology up to 4 days post AdV i.v. challenge. Applied in a respiratory AdV-C6 challenge model USC-187 manifested symptoms of toxicity [25].

2.5. (S)-HPMPO-DAPy [2,4-diamino-6-[3-hydroxy-2-(phosphonomethoxy)-propoxy] pyrimidine)

The compound anti-AdV activity registered was slightly inferior than that of cidofovir and HPMPA [26].

2.6. (S)-2242 [2-amino-7-(1,3-dihydroxy-2-propoxymethyl)purine

In vitro manifested a marked activity on AdV replication with selectivity index exceeding cidofovir [26].

2.7. Ganciclovir (GCV)

This compound is known as a drug approved for the treatment of herpes infection (cytomegalovirus infection especially) was reported to be effective against human AdVs in vitro [27]. In cell culture GCV inhibits AdV5 replication and expression of late genes [28]. These authors established a marked effect of 3% GCV in cotton rat eyes, on replication and pathology of this virus [28] Ying et al. [29] tested the GCV administered locally for prophylactic or therapeutic effect in immunosuppressed (by cyclophosphamide) Syrian hamsters intravenously infected with human AdV5 and was established that the compound suppresses AdV5 replication in the liver and AdC5-induced pathology of infected hamsters thus mitigated the consequences of the AdV infection. It was showed that GCV inhibits AdV5 DNA synthesis and late gene expression. The slight increase in GCV phosphorylation in AdV infected cells established by

Ying et al. [29] could be a result of slightly elevated cellular thymidine kinase activity, higher in testing in vivo. These authors hypothesize the direct inhibition of the AdV DNA polymerase as a possible mechanism of GCV suppressive effect on AdV DNA synthesis.

2.8. Zalcitabine (2′3′-dideoxycytidine, ddC)

This anti-HIV compound possesses a marked anti-AdV activity, even stronger than cidofovir in ocular AdV infections in laboratory animals [30].

2.9. Alovudine

Alovudine (FddT) manifest in vitro (IC_{50} = 0.2–0.7 μg/ml) and in vivo (mouse model of AdV pneumonia) anti-AdV activity of the order of that of cidofovir [26, 31].

2.10. Trifluridine (3FT) and vidarabine (Vira-A)

Anti-AdV activity of these anti-HSV compounds is moderate, and the current data on their testing are controversial [32–34].

2.11. Ribavirin (1-ß-D-ribofuranosyl-1,2,4-triazole-3-carboxamide)

This triazole nucleoside was described initially by Sidwell et al. [35]. Numerous studies were carried out on the mode of action of this compound manifesting activity toward large spectrum of viruses (predominantly RNA containing) belonging to different taxonomic groups. However, there are no data about the mechanism of anti-AdV effect of ribavirin. Several different mechanisms were formulated about the antiviral effects of this compound: (1) decreased levels of intracellular guanosine triphosphate pools based on inhibition of cellular inosine-5-monophosphate dehydrogenase; (2) inhibition of viral polymerases; (3) inhibition of RNA capping activity of viral transcripts; (4) lethal mutagenesis of the viral RNA genomes, also termed "error catastrophe," based on the induction of increased viral mutation rate over the critical error rate (especially expressed on enterovirus replication) via incorporation of the compound into newly synthesized genomes; (5) immunomodulatory role particularly on adaptive immune responses—the compound is inducer of the helper-T-cell type 1 cytokine response, but also a suppressor of the type 2 cytokine phenotype. Data about anti-AdV effect in cell culture experiments are very controversial. It was established that its activity is limited to AdVs of group C and strongly cell culture-dependent [36]. However, the plasma concentrations reached by ribavirin are 10 times below the required IC_{50} value [36, 37].

3. Nonnucleoside compounds

3.1. Abitylguanide

N′N′-anhydro-bis(β-hydroxy-ethyl)biguanide-HCl (abitylguanide) suppressed markedly the replication of a large spectrum of human AdVs, both standard laboratory strains and strains isolated from epidemic keratoconjunctivitis patients. The magnitude of inhibitory

effect varied from 1.5 to 3.8 logs. A marked correlation was established between the value of inhibitory effect and belonging of tested AdVs to various subgroups, the strongest activity being found toward viruses of subgroup C (Rosen's subgroup III). The compound susceptible period of AdV 5 replication included the total growth cycle, but is especially pronounced during the exponential phase. This was established through timing-of-addition study in primary cell cultures of human embryo kidney cells. Electron microscopy study of AdV5 morphogenesis contributed substantially to the clearing-up of the mechanism of anti-AdV action of abitylguanide. It was registered that: (1) the compound decreased about 10-fold the percentage of cells in which mature or empty virions with the characteristic nuclear localization were observed; (2) a complete absence of paracrystals; (3) the number of cells with virus particles arranged in crystals in the nucleoplasm was strongly decreased [38].

The absence of crystalline inclusions established electron microscopically in our study correlated with the established pronounced decrease of infectivity and/or lower yields of viral progeny which is in line with the meaning [39] that the protein crystals might be involved in at late steps of the virus life cycle ensuring correct capsid assembly, virus maturation and infectivity. Discussing the mode of action of abitylguanide on AdV 5 replication it has to stress on the full coincidence of the data obtained electron microscopically with the results of the timing-of-addition study demonstrating the highly-pronounced compound-sensitivity of the virus growth in the exponential phase. On the basis of the mentioned data, abitylguanide can be considered a ligand of AdV capsid protein(s) [38].

3.2. Trisubstituted piperazin-2-one derivative 15D8

This piperazinone is a result of a large screening of low-molecular substances, embracing chemical libraries of in total more than 25,000 compounds. A prospective selection of the compounds was based on protein-protein and protein-DNA interaction [40, 41]. The derivative 15D8 showed substantial anti-AdV activity (AdV 5 and AdV16 models) in dose-dependent manner at high MOI (15,000 vp/cell) with little or absent cytotoxicity at low micromolar concentration. The compound selectively inhibits AdV DNA replication in the nucleus. It is possible 15D8 to interact with viral proteins essential for DNA, including precursor of the terminal protein (pTP), AdV DNA polymerase or the DNA-binding protein (DBP). 15D8 could be considered as a potential candidate for the development of a new class of antiviral compounds to treat AdV infections [42].

3.3. Cardiotonic steroids—digoxin and digitoxin

Very surprising recently (2017), the cardiotonic steroids entered in the scope of the struggle with AdV infections. As a theoretic prerequisite of their effects were the data on dependents of AdV on the host pre-RNA splicing machinery for expression of its complete genome. On such base modulators of RNA splicing as digoxin and digitoxin could be considered as antivirals versus human AdVs. Grosso et al. [3] proved that both drugs reduced of a series of AdVs of four different species (A to D) by 2–3 logs. This is a result of affecting several steps needed for AdV genome replication (late proteins E4 or f6 and the major late capsid hexon protein is compromised). The authors proved that these two drugs altered EiA RNA splicing early in infection and partially blocked the translation from 12S and 13S to 9S RNA at later stages of

viral replication. By blocking AdV replication at one or more steps beyond the onset of E1A expression and before genome replication, digoxin and digitoxin manifest very prospective potential as antivirals for the treatment of serious AdV infections.

Convallatoxin, a synthetic cardiotonic steroid, manifested a stronger activity versus AdV5 when compared with digoxin and digitoxin. In general, these three substances alter the cascade of AdV gene expression—an effect starting after initiation of early gene expression attaining a blocking of AdV DNA replication and of viral structural proteins. These findings open a novel approach of treating AdV infections and guide the development of novel antiviral therapies.

3.4. $NMSO_3$ (sulfated sialic acid derivative)

This compound inhibits selectively cellular binding sites (sialic acid-containing receptors) of several AdV serotypes. It was established that ADVs possess this cellular tropism. $NMSO_3$ could be used for the topical treatment of ocular AdV infections [43, 44].

3.5. 2,4,5,7-tetranitro-9-fluorenone

This substance markedly inhibits the AdV cystein protease, indispensable for the production of infectious AdV virions [45, 46].

3.6. ARD-209 (ADENOVIR)

As a result of the studies carried out by G. Wadell, N. Arnberg and others in University of Umea in Sweden (2014–2017), O. Sterner and U. Ellervik (University of Lund, Sweden) synthesized the substance APD-209, announced as ADENOVIR (Pharma). It was noted that this substance with unknown structure for the publicity is considered as a new solution for the treatment of viral eye infections [47].

Other Swedish authors [48] reported also that **analogues of 2-[2-(benzoylamino)benzoyl amino]benzoic acid** possess inhibitory effect on adenovirus replication.

3.7. Short interfering RNAs

In the search for discovery of efficient anti-AdV agents it was investigated the probable impact of silencing of a set of early, middle and late viral genes on the replication of AdV5 in vitro [49]. It was established that AdV replication was inhibited by siRNAs directed against AdV E1A, DNA polymerase, preterminal protein (pTR), IVa2, hexon, and protease genes. Besides, silencing of the early and middle genes was more effective in inhibiting AdV replication than the silencing of late genes, especially sharply manifested with effect on siRNA of the DNA polymerase gene. Besides, it was found that reducing the viral genome copy numbers (AdV DNA) is a more promising strategy than the reducing the number of proteins necessary for capsid formation.

3.8. Other compounds inhibiting AdV replication in vitro

Cyclic D,L-α-peptides, cycloferon, lactoferrin nitric oxide, doxovir, heterocyclic Schiff bases of aminohydroxyguanidine tosylate, PGD peptidomimetic molecules, some medical plant substances (ref. in [17]).

4. Clinical trials

Prophylaxis with effective antivirals versus AdV EKC would be particularly useful for preventing AdV transmission to the second eye as well as to the contact persons.

Cidofovir eye drops 1% prevent severe corneal opacities in EKC patients, dose-dependent local toxicity at frequent administration been registered [50, 51]. This anomalous nucleoside at concentration of 1% applied topically in a combination with 1% cyclosporine demonstrated therapeutic effect on patients with EKC in a controlled clinical pilot study [51]. No placebo-controlled randomized trials have been carried out on immunocompromised patients.

Controversial results were obtained with cidofovir the treatment of AdV infections in children undergoing bone marrow or stem cell transplantation [52]. Evidence was accumulated that as earlier AdV infection is detected for starting cidofovir treatment the better curative results were registered. Although cidofovir exhibits antiviral activity versus all AdV species, it possesses low oral bioavailability and significant toxicity (tubular necrosis) and does not confer long-term protection [53].

However, the lipid conjugate of cidofovir, brincyclovir (BCV; hexadecyloxy propyl-cidofovir; CMX001) is currently in Phase II clinical trial [25, 54], unfortunately manifesting a significant toxicity to the kidney and gastrointestinal tract.

The topical ganciclovir application against EKC [55] merits special attention. In a published clinical study, treatment with 0.15% GCV ophthalmic gel improved outcome of AdV conjunctivitis [56]. Three other clinical trials evaluating 0.15% GCV ophthalmic gel were organized, two of them finished: (1) Efficacy and Safety of GV 550 in Acute Adenovirus Keratoconjunctivitis (Clinical Trials.gov. trial NCT01156025) and (2) Efficacy and Safety of GV 550 in Acute Adenovirus Keratoconjunctivitis (a Clinicaltrialsregister.eu trial). Both included placebo and treatment groups on 40 persons each (in fact Phase II of clinical trial). In the third trial, Clinical Trials.gov trial NCT1533480 (A Placebo Controlled Comparison of Topical ZIRGAN Versus Genteal for the treatment of Adenovirus Conjunctivitis) which is currently in course, GCV is administered topically as 0.15% gel (ZIRGAN®) compared with 0.3% Hypromellose gel (Genteal gel®), serving as placebo (Phase IV).

On the base of the promising results with povidone-iodine (PVP-I), a microbicidal agent possessing also virucidal properties [57], topical ganciclovir and PVP-I combination drops have shown the most recent potential, but both therapeutics need to be investigated in larger scale studies [58].

The experimental data in vivo are in favor of GCV to be considered as an option for the treatment of AdV infections in immunocompromised patients.

Ribavirin efficacy for the treatment of AdV infections was very controversial [36, 59].

N-chlorotaurine (a week oxidant) manifested effectivity in phase II clinical trials with viral conjunctivitis [60].

During a severe outbreak of EKC caused by AdV 8 in 1972–1973 in Bulgaria abitylguanide was tested in two double-blind, randomized trials, carried out on total 349 patients (trial 1–151 patients; trial 2–198 patients) with virologically confirmed diagnosis.

Abitylguanide was applied as 1% eye drops (in saline). In each of the two trials, patients were divided in three groups: group I placebo (patients with symptomatic treatment), group II -abitylguanide 1% + symptomatic treatment, group III—patients treated with abitylguanide 1% only. Curative effect of abitylguanide was almost identical in group II and III in both trials. Moreover, the drug had a preventive effect on infection of the second eye. The abitylguanide treatment exerted a marked curative effect on the severity and duration of the disease: (1) more than twofold decrease in both trials in the number of patients with EKC form associated with keratitis; (2) five- and sixfold decrease in trial 1 an trial 2, respectively, in the incidence of severe keratitis; (3) two- and fivefold decrease in trial 1 and trial 2, respectively, in the number of patients with impaired vision; (4) twofold decrease of the healing time [61–63]. Effectivity toward AdV-induced EKC of the drug applied topically was confirmed in series of placebo controlled trials (not following the double-blind scheme) in three other ophthalmic clinics in the country, carried out in the second half of the 1980s. Pencheva et al. [64] in the Varna Medical Faculty registered marked decrease of the patients number with keratitis, two-fold shortening of the healing time in abitylguanide treated patients, affection of the second eye—80% in the placebo group, and 22.6% in abitylguanide treated group.

In preliminary carried out study abitylguanide manifested a very high local tolerance (1, 2 and 3% eye drops in saline) tested in 21 volunteers (in rabbits—till 20% eye drop).

The pronounced effect of abitylguanide [65] in abovementioned trials on EKC patients served for the development and implementation in pharmaceutical industry of preparative ADENOSTATIN COLLYRIUM® (Pharmachim Ltd., Sofia) which clinical use marked favorable estimation by ophthalmologists in this country (tested in Japan, as well).

The clinical use of cardiotonic steroids as anti-AdV agents needs special consideration. As digitoxin has been associated with toxicity, the use of cardiac glycosides as antivirals would be short term, in contrast to the chronic use in patients with heart diseases. Having in mind that anti-AdV agents have to be used for the treatment of severe respiratory and disseminated diseases, these drugs seem more attractive as potential agents for the topical treatment of EKC and even for the prophylaxis of persons contact to EKC patients [3].

There is no doubt that chemotherapy of AdV infections occupies leading position as a tool for anti-etiological treatment. Therefore, the development of effective anti-AdV agents is especially a big task of the scientists and clinicians. The above-presented panorama of antivirals versus AdVs and AdV-caused infections shows that a lot of work is done for the realization of this problem. The author would like to mention the main directions that determined the development of antivirals and their implementation in the clinical practice: (1) discovery of the targets in virus growth cycle for chemotherapeutic attacks—a lot of "wide" places could be pointed in the AdVs; (2) attainment in the organic chemistry—modeling of new effective molecules with anti-AdV effects among anomalous nucleosides, end especially of non-nucleoside compounds ligands of AdV proteins; (3) development of adequate methodology for antiviral testing starting from the initial in vitro screening, and application of purified AdV structural and eventually nonstructural proteins as cell-free systems (approach contributed substantially for the successful development of anti-hepatitis C drugs; as concerns the in vivo testing, in the last years, several very convenient and adequate models were described and successfully used

(more precisely ocular AdV infections in laboratory animals); (4) application of methods for express diagnostic of ADV infections, in order earliest start of the respective treatment with anti-AdV chemotherapeutic agents. More detailed consideration of this topic was presented by Kaufman [66] and Luchs [67].

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