AN EFFECTIVE AND SAFE LINKER IS A KEY TO THE SUCCESS OF AN ANTICANCER CONJUGATE

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Cancer is a leading cause of death, accounting for approximately 10 million cases or one in six deaths in 2020. For decades, chemotherapy with cytotoxic agents such as DNA analogues (5-fluorouracil and 8-azaguanine), DNA alkylating agents (cisplatin and actinomycin D), antimetabolites (aminopterin and methotrexate) and tubulin inhibitors (paclitaxel derivatives) has remained the main approach to cancer treatment and vincristine). However, for most of these chemotherapeutic agents, the therapeutic index is very low, and due to the drug's effect not only on tumor cells, but also on healthy cells, serious side effects often occur.

In recent years, the search for new targeted antitumor drugs has been actively pursued. The main mechanisms of their action are specific effects on key signaling pathways important for the cancer cell functioning, or targeted delivery of a cytotoxic agent to the tumor using nanoparticles or vectors that can penetrate and/or accumulate in cancer tissue [1].

A typical targeted chimeric conjugate consists of three main parts:

- a vector whose task is to deliver the molecule to the tumor;
- a highly active cytotoxic substance that causes the death of tumor cells;
- a linker that combines the vector and the toxin into a single molecule.

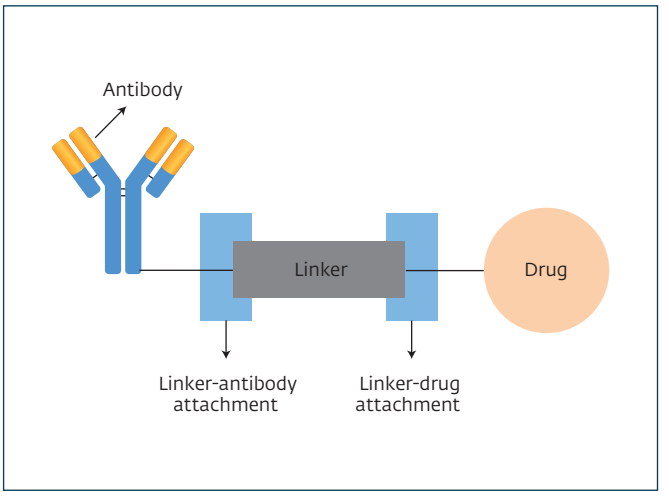

Fig.1. *Structure of the ADC molecule*

The linker must be stable during circulation, but release a cytotoxic substance when it enters the cancer cell. Since anti-tumor conjugates use very active compounds, any premature release leads to serious toxic effects [2]. The chemical synthesis of linkers is quite complex, as is the choice of its nature which in turn determines the release profile of the cytotoxin. Linker stability influences the overall pharmacokinetic (PK) properties, toxicity, and therapeutic indices of anti-tumor conjugates [3].

Among various anti-tumor conjugates, antibody-drug conjugates, or ADCs (Fig.1), are the most developed chimeric molecules. By 2023, more than dozen ADCs were approved for clinical use, over 200 are studied in clinical trials.

Besides ADCs, linkers are used in nanotechnology for anticancer therapy. In Fig.2 numerous delivery systems that use linkers to target tumors are shown.

CLEAVABLE VS NON-CLEAVABLE LINKERS

Linkers used in chimeric conjugates can be divided into two main groups depending on the mechanism of cytotoxic drug release: cleavable and non-cleavable. The former release a cytotoxic substance in response to certain stimuli – pH, redox status, levels of certain enzymes,

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hypoxia and the presence of reactive oxygen species, which distinguish cancer and healthy cells. Non-cleavable linkers release the toxin only after the entire chimeric molecule has been degraded.

Cleavable linkers are versatile and their behavior is predictable, but there is a danger of premature cleavage. Non-cleavable linkers are safer and more stable in systemic circulation, but release of the cytotoxic drug occurs intracellularly due to lysosomal degradation of the antibody [4]. Their effectiveness is based only on the preferential accumulation of the conjugate in the tumor due to their affinity for certain receptors or the enhanced permeability and retention effect (EPR-effect), and not on selective cleavage in the cancer cell. Of the registered drugs, only T-DM1 (Trastuzumab emtansine) has a non-cleavable linker.

Cleavable linkers are believed to be suitable for conjugates targeting solid tumors, and non-cleavable linkers – for the treatment of hematological cancers. However, this is not always true. For example, two conjugates containing the same antibody (cAC10) and cytotoxic agent (MMAF) and differing only in the type of linker (cleavable valine-citruline (Val-Cit) linker vs non-cleavable thioester linker) were tested in vitro and in vivo against a panel of solid cancers and showed similar activity (Fig.3) [5].

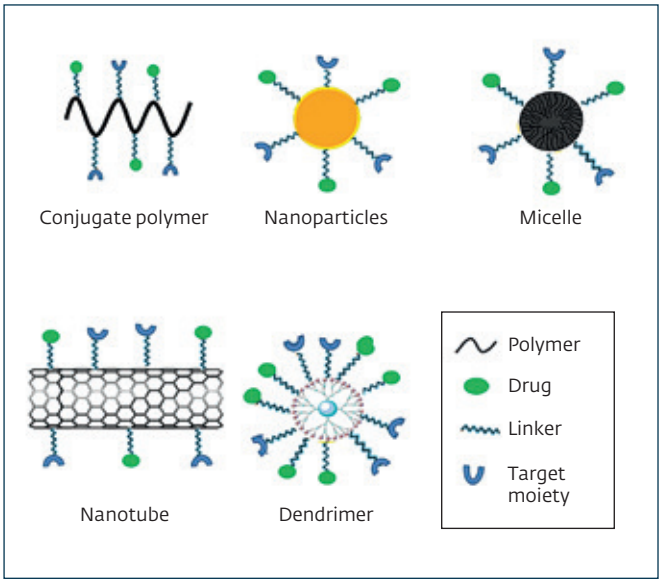

Fig.2. *Numerous drug delivery systems using linkers for tumor targeting*

MALEIMIDOCAPROYL LINKER

The most common examples of non-cleavable linkers are the thioether linkers, N-succinimidyl-4-(N-maleimidomethyl)cyclohexane-1-carboxylate (SMCC) and maleimidocaproyl (MC). There are many more cleavable linkers. There are three main types of chemically cleavable linkers in common use today: acid-cleavable, enzyme-cleavable, and disulfide-reducible. At the same time, there is an active search for safe linkers, the cleavage of which is associated with such distinctive properties of a cancer cell as low levels of molecular oxygen and high levels of reactive oxygen species, as well as linkers capable of releasing the active component in response to irradiation with light or X-rays. This article focuses on the cleavable type of linkers.

ACID-CLEAVABLE LINKERS

The acid-cleavable linkers were the first ones which were used in early ADCs. They take advantage of the pH difference between tumor tissue and plasma. The pH of tumors is acidic, which is associated with dysregulation of acid-base homeo-

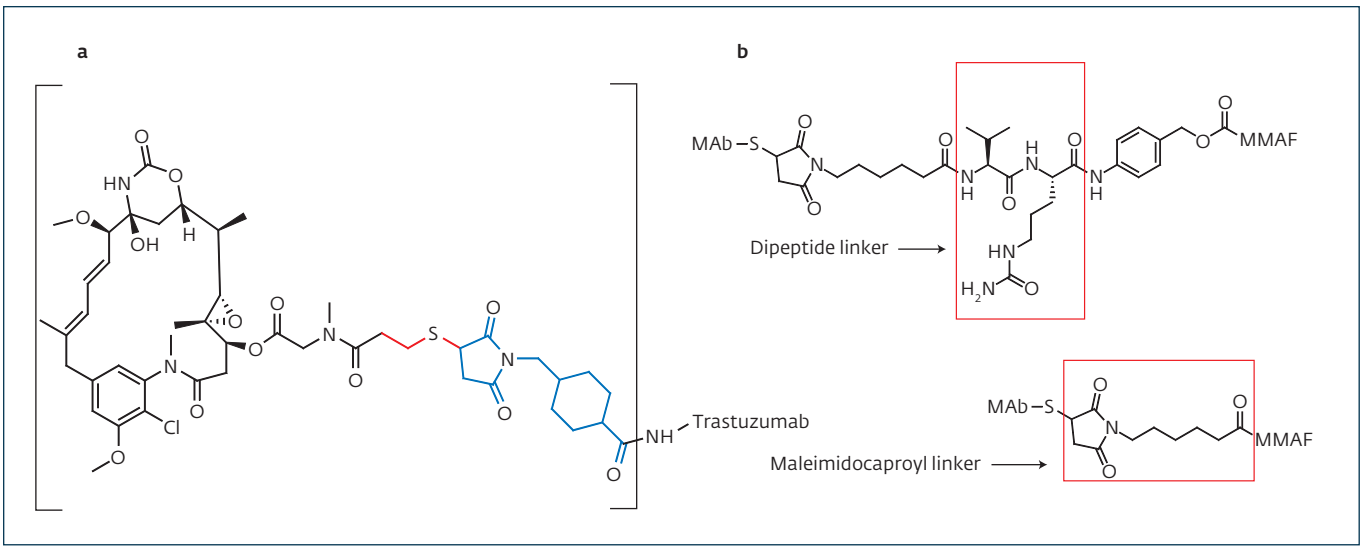

Fig.3. *a – Structure of the drug T-DM1, b – ADC conjugates with different types of linkers*

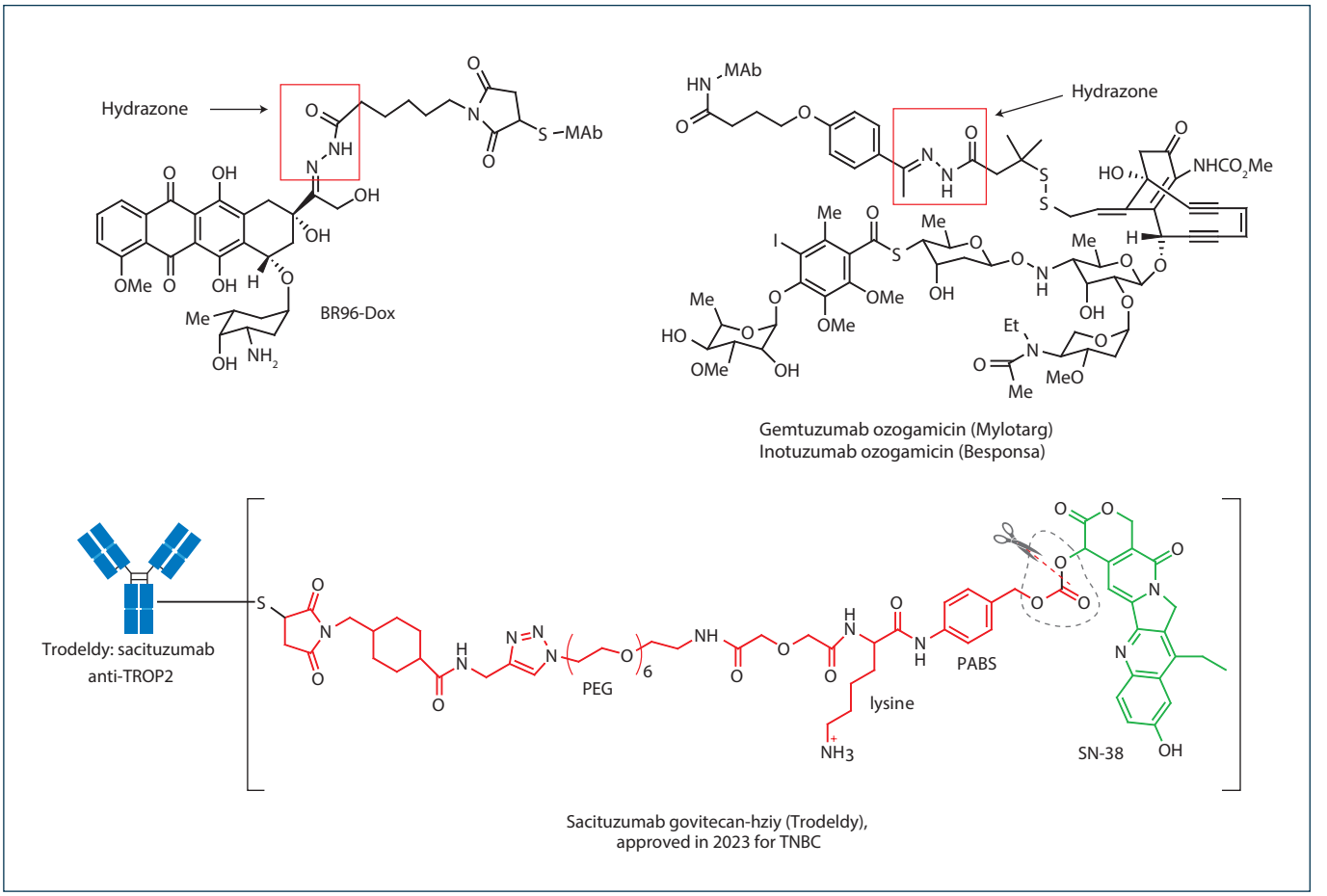

Fig.4. *Approved ADCs with acid-cleavable linkers*

static mechanisms. Tumor cells are well adapted to an acidic microenvironment [6, 7]. In vitro studies show that maximum tumor cell proliferation occurs at pH 6.8.

The difference between the acidity in human plasma (pH 7.4) and the tumor microenvironment (6.5–6.9) is used to create prodrugs capable of releasing the active component exclusively under low pH conditions – chimeric conjugates, micelles and nanoparticles. In most cases, a hydrazone or carbonate linkers are used, however, work continues on the creation of linkers containing acetals, ketals, silyl ethers, etc.

In general, acid-cleavable linkers are not suitable for conjugating highly cytotoxic drugs, and their low stability limits their widespread use in ADC design. A key example is the drug BR96-Dox, a conjugate of doxorubicin with cysteine residues of a chimeric monoclonal antibody connected via a hydrazone linker targeting the Lewis Y antigen (Le(Y)), the expression of which is characteristic for gastrointestinal tumors.

Hydrazone-based acid-cleavable linkers are currently used in two approved ADCs [8]. Mylotarg (Pfizer Inc., Brooklyn, NY, USA) was the first approved ADC for the treatment of acute myeloid leukemia, but was discontinued in 2010 due to linker instability; however, in 2017, the drug reappeared on the market with updated treatment regimen recommendations due to its high dose-dependent hepatotoxicity.

The acid-sensitive N-acylhydrazone linkage was also used in Inotuzumab ozogamicin (Besponsa), approved in 2017. An in vitro stability study of the Besponsa linker showed that hydrazone degradation occurs in the circulatory system at a rate of only 1.5–2% per day [9]. However, other hydrazone-containing ADC linkers have demonstrated a discrepancy between stability in buffer and stability in plasma. The reasons for the increased rate of hydrolysis in plasma remain unclear, and the highly variable stability profile of hydrazones compared to other cleavable linkers prevents their use.

An acid-cleavable carbonate linker is present in the ADC Sacituzumab govitecan-hziy (Trodeldy), approved in 2023 for the treatment of triple-negative breast cancer (TNBC) (Fig.4).

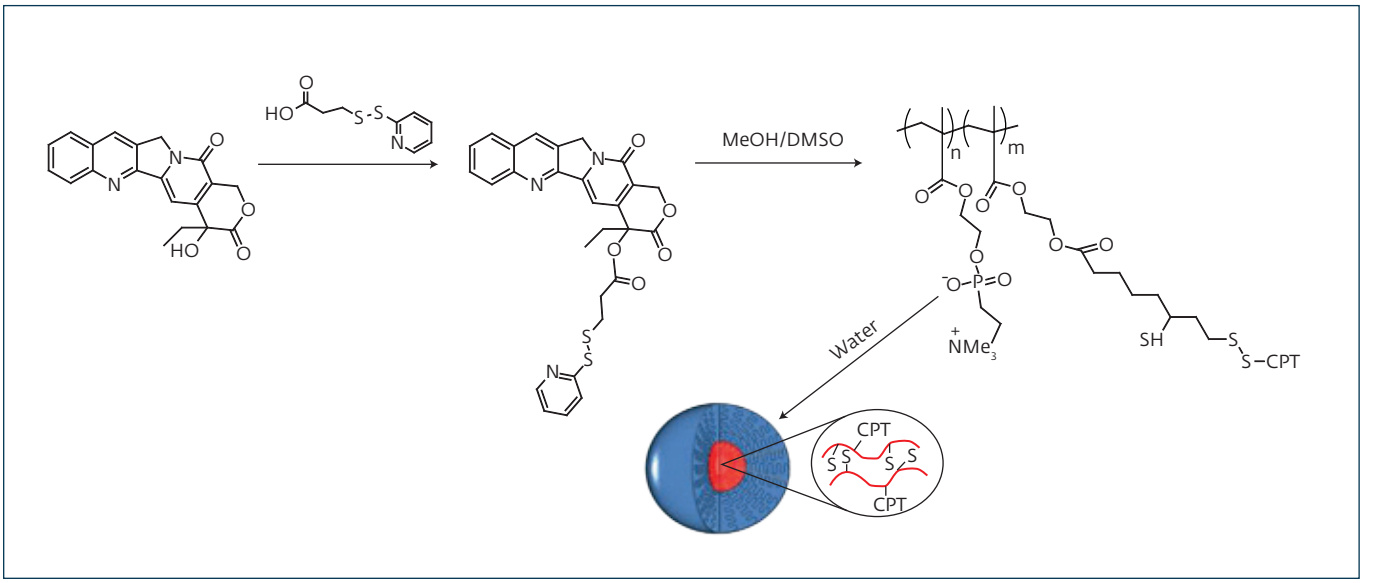

Fig.5. *Synthesis of CPT-pyridyl disulfide, conjugation with poly(MPC-DHLA) and formation of cross-linked micelles*

However, despite the successful use of acid-cleavable linkers in clinical practice, they cannot provide reliable safety. For this type of linker, only compounds with moderate cytotoxicity are applicable. Highly potent cytotoxic agents, which are now preferred for ADCs, require more stable linkers.

DISULFIDE LINKERS

The environment in cancer cells has reductive properties, primarily due to the high level of glutathione (GSH), a natural tripeptide antioxidant containing a free thiol group. The intracellular level of GSH in a cancer cell is 1–10 mM, which is 1000 times higher than its level in normal cells (2–20 μM) [10]. This property is the basis for the work of the disulfide linker, which selectively releases the cytotoxin when it enters the tumor environment.

For efficient delivery of various bioactive molecules, a disulfide linker can be included in the main chain [11] or side chain [12] or in the dendritic structure [13].

Disulfide bridges are actively used to construct various prodrug micelles, for example, camptothecin (CPT) was successfully conjugated to a polymer scaffold based on 2-methacryloyloxyethylphosphorylcholine (MPC) and dihydrolipoic acid (DHLA) via a disulfide bridge. The micelles showed a decrease in the release rate of CPT compared to physically encapsulated CPT (Fig.5)

Doxorubicin was also covalently conjugated to folate enabling tumor targeting using a disulfide linker. Following folate-mediated cellular uptake of the conjugate, the disulfide linkers were cleaved due to elevated GSH levels and released doxorubicin. The prodrug showed significant cytotoxicity against the A549 and MCF 7 tumor lines [14] (Fig.6).

Examples of successful use of a disulfide linker include Elahere (mirvetuximab soravtansine-gynx), approved in 2022 for the treatment of FRα-positive ovarian and fallopian tube cancer, and Lorvotuzumab mertansine (IMGN901), undergoing clinical trials in patients with small cell lung cancer and ovarian cancer.

The activity and pharmacokinetic properties of conjugates depend not only on the type of linker, but also on the chemical environment of the cleavage site. Thus,

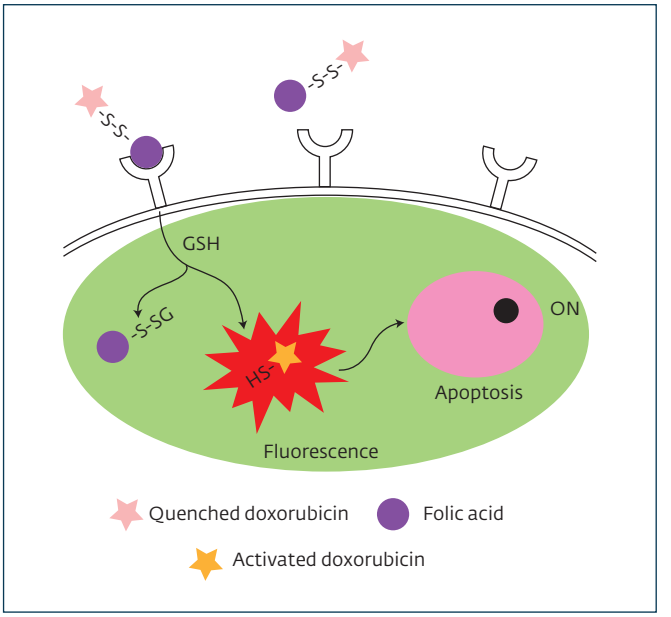

Fig.6. *Cytotoxic effect of a drug after disulfide bond cleavage in tumor cells*

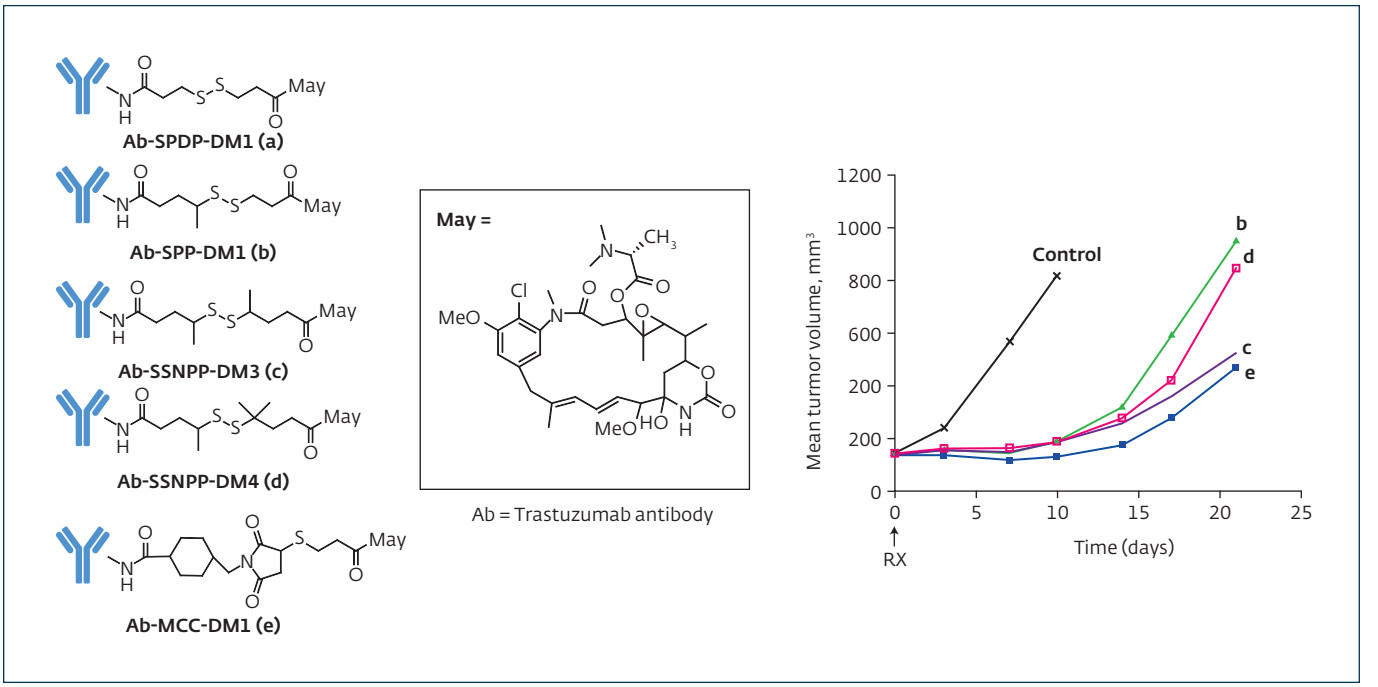

Fig.7. *Structures of trastuzumab-maytansinoid conjugates with disulfide linker (a-d) or thioether linker (e) and comparison of their in vivo potency*

the authors of [15] synthesized a series of ADCs containing exactly the same antibody, active component and disulfide linker, but differing in the number of methyl groups next to the linker. A substance with a non-cleavable thioether linker was used as a reference drug. Fig.7 shows that while conjugates with a noncleavable linker and a linker containing three methyl groups were approximately equipotent in the xenograft mouse model, compounds containing one or two methyl groups exhibited significantly lower activity.

The stability of the disulfide linker in plasma and in a reductive medium, simulating the meduim inside a cancer cell, also differs significantly depending on the chemical environment: the more sterically hindered the disulfide moiety, the higher its stability [16].

ENZYME-CLEAVABLE PEPTIDE LINKERS

During cancer growth, certain proteases are overexpressed, and the levels of some of them, such as cathepsin B, differ significantly between cancer and normal cells (Fig.8). This feature made it possible to develop a number of peptide linkers cleaved by cathepsin B – valine-citruline (Val-Cit), phenylalanine-lysine (Phe-Lys), valine-alanine (Val-Ala), etc. To facilitate the approach of the bulk enzyme to the cleavage site in a self-destructing spacer is additionally introduced into the molecule, for example, p-aminobenzylcarbamate (Fig.8) [17].

The most commonly used dipeptide linker, Val–Cit, is used in the drugs Adcertis (Brentuximab vedotin, 2011, recurrent Hodgkin lymphoma (HL) and relapsing systemic anaplastic large cell lymphoma), Padsev (enfortumab vedotin-ejfv, 2023, bladder cancer), Polivy (Polatuzumab vedotin, 2023, diffuse large B-cell lymphoma), Tivdak, (tisotumab vedotin-tftv, 2021, cervical cancer), Aidixi (Disitamab vedotin, 2021, stomach cancer) and a number of other drugs currently in clinical trials (Fig.8).

However, despite the successful use of this linker in clinical and preclinical practice, it has a number of disadvantages that complicate the development of chimeric molecules and cause undesirable side effects.

One of the limitations of preclinical studies of drugs with this linker is its instability in mouse and rat plasma due to the presence of a specific rodents protease, carboxylesterase 1C (Ces1C). Although Val-Cit is stable in human plasma, the linker is rapidly degrade in plasma of mice, which are used in most xenograft models.

Another disadvantage of the Val-Cit linker is its low specificity to the action of cathepsin B [18]. Using methods to suppress the expression of cathepsin B through deletion of the CRISPR-Cas9 gene or knockdown of shRNA, cells similar to KPL-4 cells (expressing receptors for HER2) and WSU-DLCL2 (expressing receptors for CD22), but not containing cathepsin B, were obtained. It was demonstrated that the inhibition of proliferation of KPL-4, WSU-DLCL2 (3500 CD22) cells and corresponding cathepsin B-deficient cells after treatment with ADCs containing the cytotoxin MMAE coupled to HER2 or CD22 antibodies via Val-Cit is essentially the same and doesn't depend

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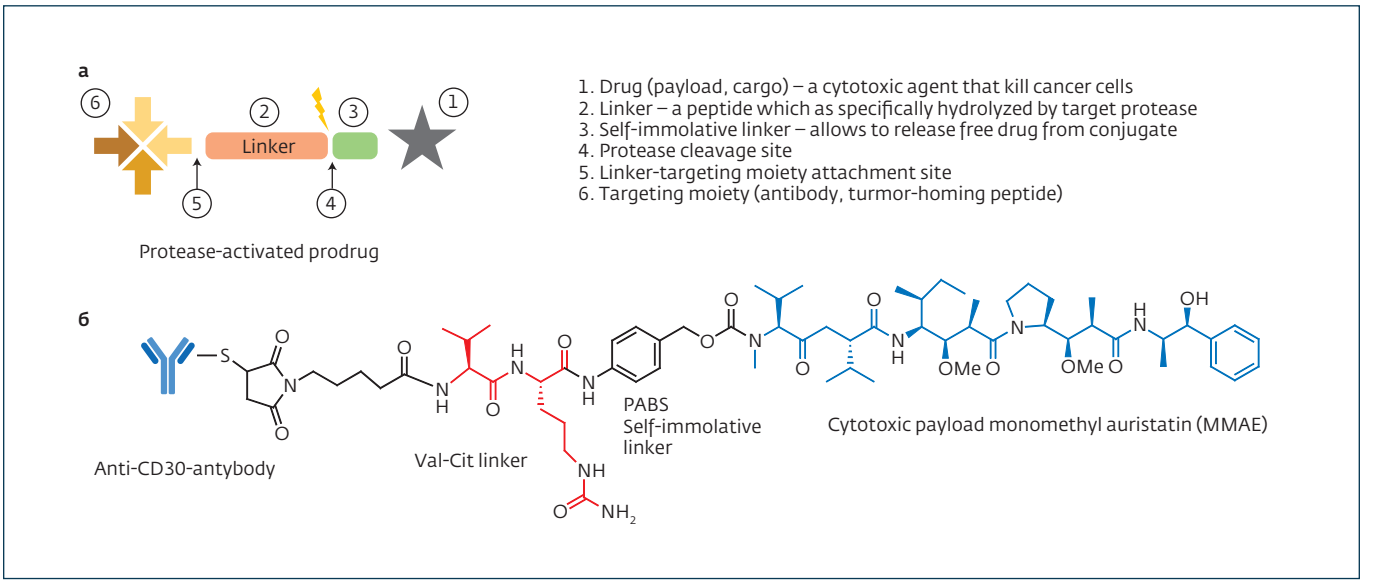


Fig.8. *a – The main structure of ADC. The key components are the cytotoxic payload, linker and targeting moiety. When the peptide bond is hydrolyzed by a protease, the free payload is released. b – Structure of brentuximab vedotin. The protease cleavable linker (Val-Cit, VC) is marked in red and the cytotoxic load (MMAE) is marked in blue*

onthe level of cathepsin B. This means that the linker can be cleaved not only by cathepsin B, but also by other enzymes, resulting in indiscriminate release of the cytotoxic substance and off-target toxicity. Research into the nonspecificity of the Val-Cit linker is currently ongoing.

Table 1. *Types of proteases expressed during turmor development*

Stage	Expressed proteases
Growth	Cathepsin L Kallikrein 3 and 10 Prostasin MMP-9
Survival	Caspases MMP-3 USP7
Angiogenesis	Neprilysin ADAM- proteases MMP-9, MMP-12, MMP-19 Kallikreins
Invasion	MMP DPP4 Cathepsin B Cylindromatosis (CYLD) Matriptase Hepsin

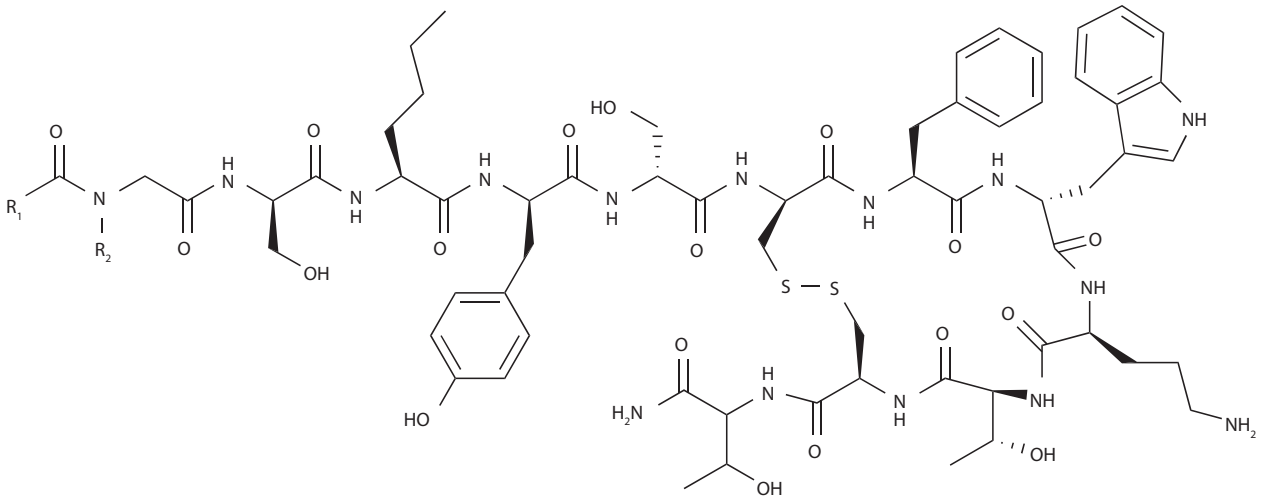
CARBAMATE LINKERS

Compounds containing carbamate bonds are stable under neutral pH conditions, but are prone to acid-catalyzed hydrolysis. Due to their special chemical properties, carbamate linkers are widely used in drug delivery to achieve high efficiency [19]. The most common approach to preparing compounds with carbamate linkers is an addition reaction via an isocyanate and an alcohol hydroxyl. The metabolic stability of the carbamate group is higher than the ester group, depends on the nature of the hydroxyl and amine fragments and decreases in the following order: Ar-OCO-NH-Alk>>Alk-OCO-NH-Alk~Alk-OCO-$N(Alk)_2$≥Alk-OCO-N(endocycle)≥Ar-OCO-$N(Alk)_2$~Ar-OCO-N(endocycle)≥Alk-OCO-NH-Ar~Alk-OCO-NH-Ac Alk-OCO-NH_2>cyclic carbamates [20]. Although the carbamate moiety is used in prodrugs to improve bioavailability or physicochemical properties, it is often found in combination with additional functional groups in anticancer conjugates [21].

Carbamate linker with built-in nucleophile

In this type of linker, a nucleophile is located in spatial proximity to the carbamate group, and can attack the carbon atom of the carbonyl group, releasing the cytotoxin.

In [22], the authors studied the influence of the type of nucleophile, the length of the spacer between the nucleophile and the carbonyl group, and the nature of the hydroxyl group on the antiproliferative activity and kinetics of release of the active component from the conjugate



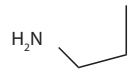
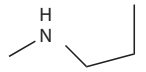
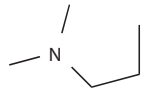
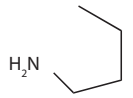
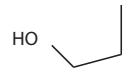
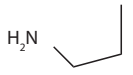
	Cytotoxicity IC_{50} IMR-32 Cells
	374±18
	54,2±6
	571±48
	>1000
	>1000
	2,79±0,33
Camptothecin	2,21±0,44
Combretastatin	4,36

Fig.9. *Structures of camptothecin and combretastatin conjugates containing various types of built-in nucleophiles and their cytotoxic activity*

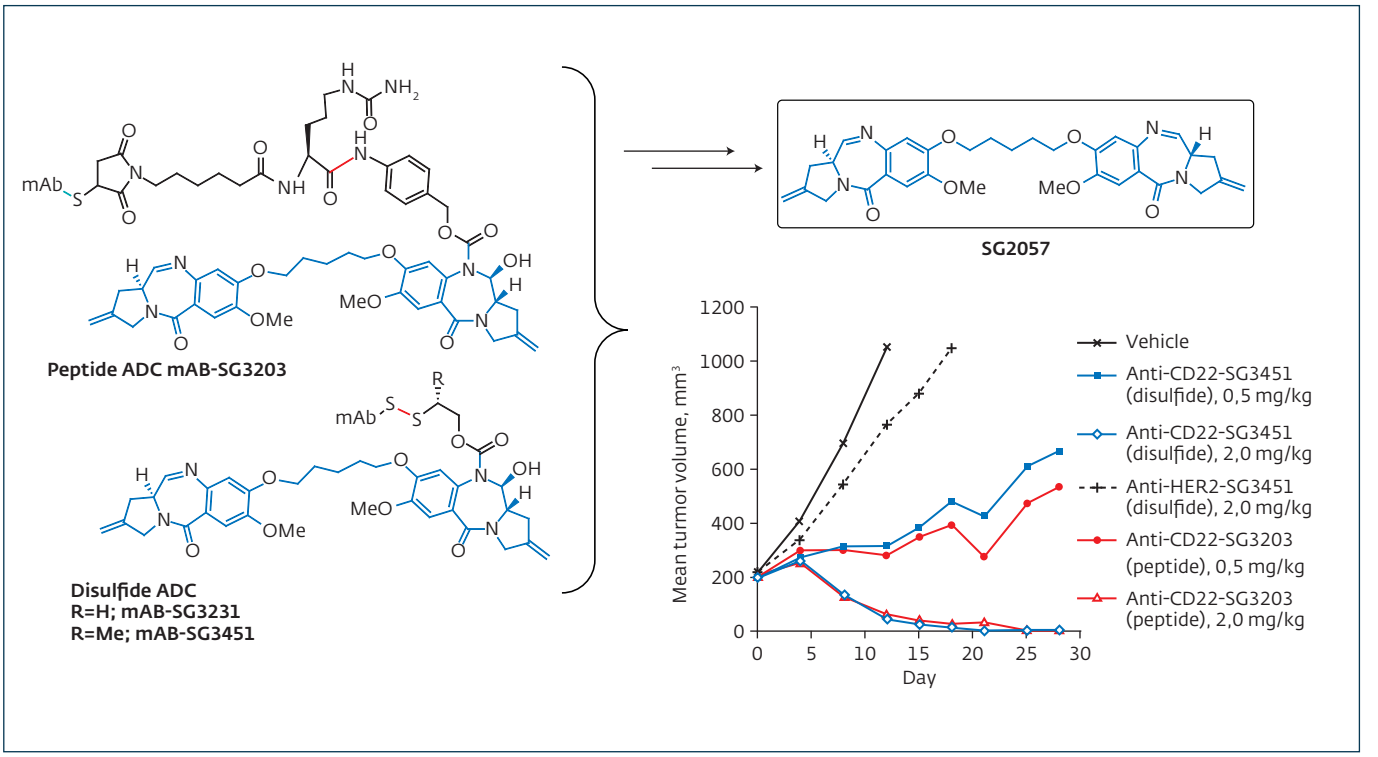


Fig.10. *Comparison of the effect of linkers on cytotoxic activity for two ADCs*

including a somatostatin analogue and two known cytotoxic agents: combretastatin and camptothecin. Camptothecin is a topoisomerase I inhibitor, containing a tertiary hydroxyl group at the C-20 position (Fig.9), its pKa value is approximately 18. Combretastatin is a tubulin-binding agent, with a phenolic hydroxyl group, its pKa value is 10.3. It turned out that the conjugate containing combretastatin has the same cytotoxic activity as the original combretastatin. The addition of camptothecin to the vector reduced its cytotoxic activity by approximately 20 times. Moreover, both the activity and the kinetics of cytotoxin release significantly depended on the type of nucleophile. The stability of the linker associated with the phenolic fragment (6) turned out to be 4.5–26.5 times lower than the stability of the linkers associated with the tertiary alcohol fragment (Fig.9).

Conjugates often contain a combination of disulfide and carbamate moieties separated by an ethylene spacer. A comparative study of the classic Val-Cit linker and a combination of disulfide and carbamate linkers is presented in [23] (Fig.10). After cleavage of disulfide linker in reductive medium of tumor the SH-nucleophile formed attacks a carbamate carbonyl leading to cytotoxin release. The authors studied two ADCs, the only difference between which was the nature of the linker, in a rodent xenograft model. It turned out that regardless of the type of linker, their effectiveness in suppressing tumor growth was approximately the same. A safety study in rats showed that the maximum tolerated dose of the conjugate containing the linker combination was higher than that of the Val-Cit linker.

A similar approach was used in carbamate linkers, where the active component was attached not through the hydroxyl group, but through the amine group. An example of such a conjugate is Cybrexa Therapeutics' solid tumor compound CBX-12. CBX-12 includes a highly potent topoisomerase I inhibitor that is in the same class as the payloads used by the ADC conjugates ENHERTU® and TRODELVY™. Unlike these ADCs, CBX-12 contains a specific pHLIP peptide and is able to target cancer cells regardless of the expression of any antigen, thereby expanding the target patient population. In the test compound, exatecan is attached to the peptide vector through an amino group, and the linker contains a combination of carbamate and disulfide fragments. The compound is currently undergoing clinical trials [24].

Carbamate linker with radiation activation

In general, it is challenging to achieve highly localized and controlled drug activation deep in tissue in a non-invasive manner. Techniques such as heat, ultrasound and laser irradiation cannot easily penetrate deep into tissue. In contrast, ionizing radiation is capable of passing through

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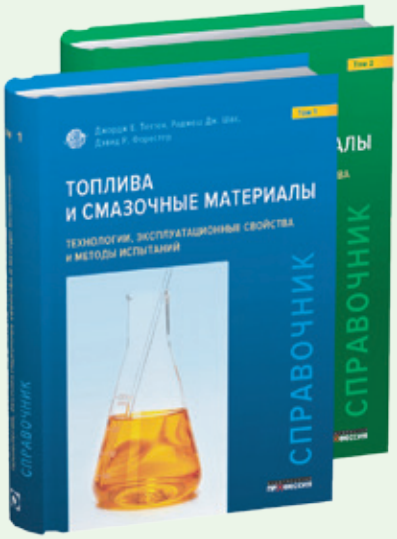

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Рассмотрены все основные нефтяные жидкости и продукты: топлива, нефтяные парафины, нефтяной газ; базовые, моторные, турбинные и компрессорные масла; пластичные смазки, присадки, жидкости для теплопереноса и металлообработки. Уделено внимание «не-нефтяным» материалам – синтетическим смазкам, растительным маслам и ионным жидкостям, а также их использованию в качестве альтернативы нефтяным. Отдельные разделы посвящены методам определения свойств и эксплуатационных характеристик материалов с использованием современных аналитических методов: хроматографии, ИК-, масс- и ЯМР-спектрометрии; экологическим характеристикам топлив и смазок; основам теории смазывания. Обширный справочный иллюстративный материал имеет практическую направленность, качественно дополняет описание и закрепляет выводы авторов.

Справочник написан ведущими экспертами ASTM International.

Впервые этот справочник был издан в 2003 году и содержал 38 глав. Настоящее, расширенное и дополненное издание, содержит 49 глав, включая обзоры методов производства топлив, масел, смазок, ионных жидкостей, а также методов их испытаний. В обновленном издании большинство глав существенно углублены и дополнены, а некоторые главы полностью переписаны.

Задача справочника – предоставить глубокое, всестороннее руководство по топливам и смазочным материалам на основе оригинальных публикаций-первоисточников.

Данный справочник продолжает традицию всеобъемлющего источника как академических, так и практических знаний. Мировой опыт, собранный в одной книге, будет необходим специалистам и полезен студентам нефтяной отрасли.

По широте охвата, глубине и подробному изложению рассматриваемых вопросов справочник не имеет аналогов в России и послужит надежным современным источником академических и практических знаний для специалистов, разработчиков, исследователей в области нефтепереработки, нефтехимии и в смежных областях.

Основные разделы справочника

Часть I. ПРОЦЕССЫ ПОЛУЧЕНИЯ ТОПЛИВ И СМАЗОЧНЫХ МАТЕРИАЛОВ ИЗ НЕФТИ
Часть II. ТОПЛИВА: ФУНКЦИИ И СВОЙСТВА
Часть III. УГЛЕВОДОРОДЫ И СИНТЕТИЧЕСКИЕ СМАЗОЧНЫЕ МАТЕРИАЛЫ: ФУНКЦИИ И СВОЙСТВА
Часть IV. МЕТОДЫ ОПРЕДЕЛЕНИЯ СВОЙСТВ И ЭКСПЛУАТАЦИОННЫХ ХАРАКТЕРИСТИК

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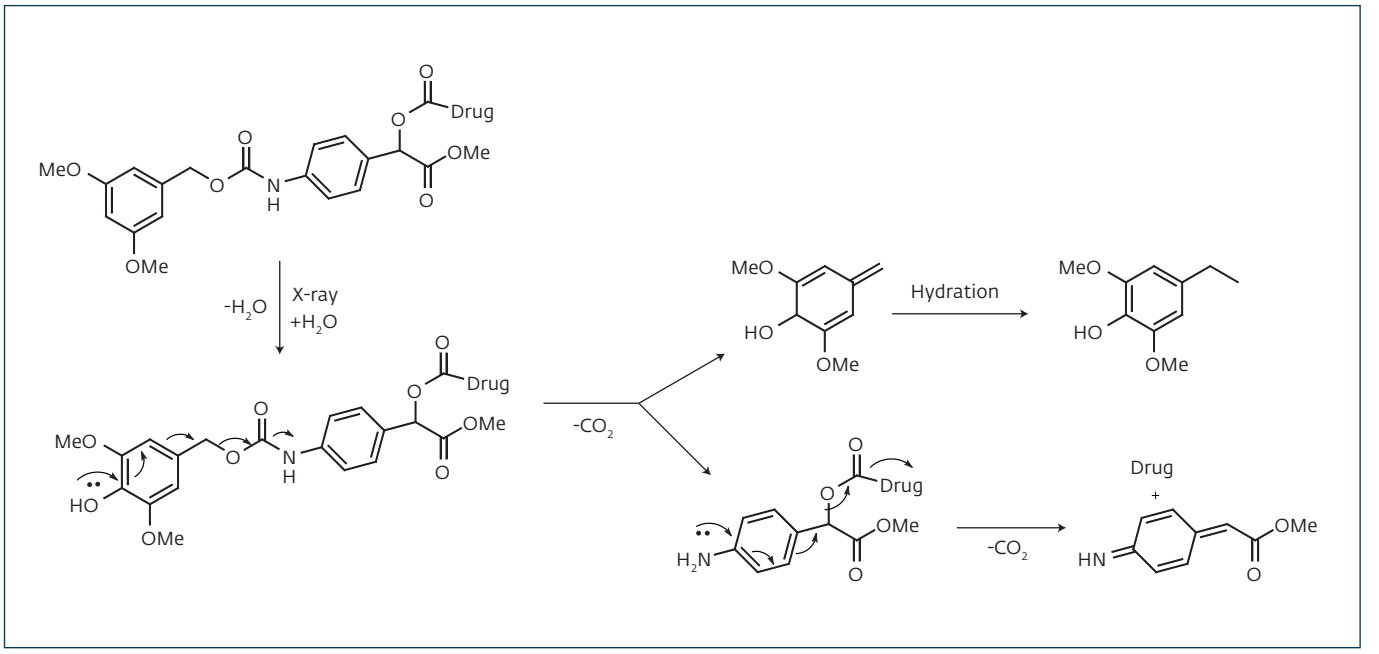

Fig.11. *Radiation-induced radical hydroxylation with sequential 1,6-elimination and loss of SIL moiety releases drug payload*

tissue through focused beams of gamma, proton and x-ray radiation. Cellular prodrugs that are activated by ionizing radiation are known for small molecules and with suboptimal drug release [25, 26]. A prodrug containing a cytotoxic payload tethered via self-immolative linker to a protein conjugation anchor, and a radiation-activated trigger was studied in [27]. In this type of activated linker, the active component can be attached to the vector through either a hydroxyl or an amino group, as has been demonstrated with human albumin conjugates with MMAE and doxorubicin.

Combining these components together produced Alb-DMBA-SIL-MMAE and Alb-DMBA-SIL-DOX as radiation-activated albumin-drug conjugates. Under the influence of X-ray irradiation in clinically acceptable doses (8 Gy), radical hydroxylation of the terminal 3,5-dimethoxybenzyl group occurs, which causes subsequent rearrangement with the release of the active component (Fig.11).

The advantage of the proposed linker is the ability to localize the effects of ionizing radiation, releasing toxic substances only in the tumor area, thereby reducing the risk of off-target toxicity. In addition, there is a possibility that radiation and the cytotoxic agent have a synergistic action, enhancing the antitumor effects of each.

ROS-responsive linkers

Elevated levels of reactive oxygen species (ROS) are observed in a number of pathophysiological conditions, including can-

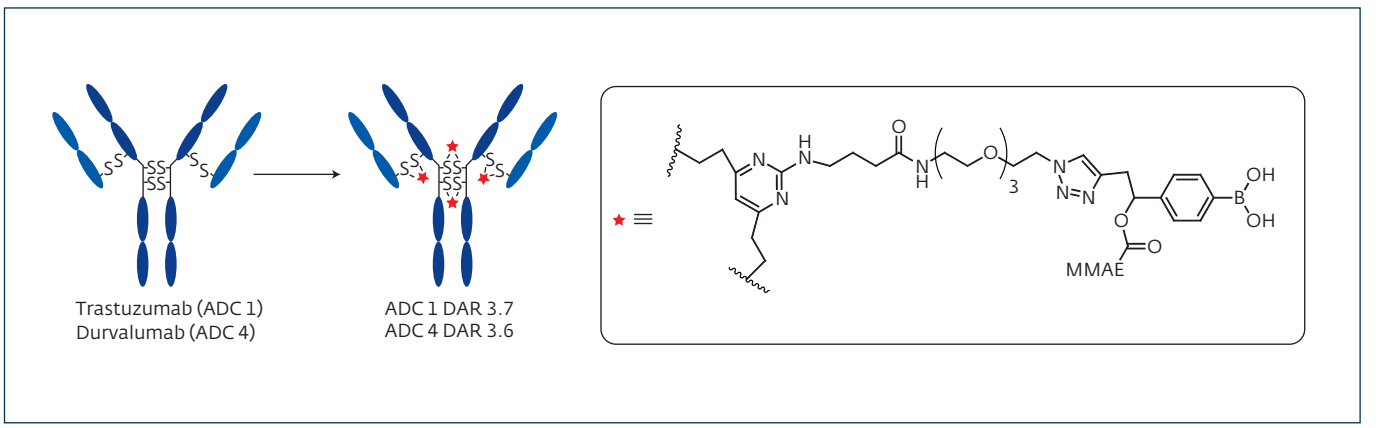

Fig.12. *Synthesis of MMAE conjugates with trastuzumab and durvalumab containing a linker with a phenylboronic acid fragment*

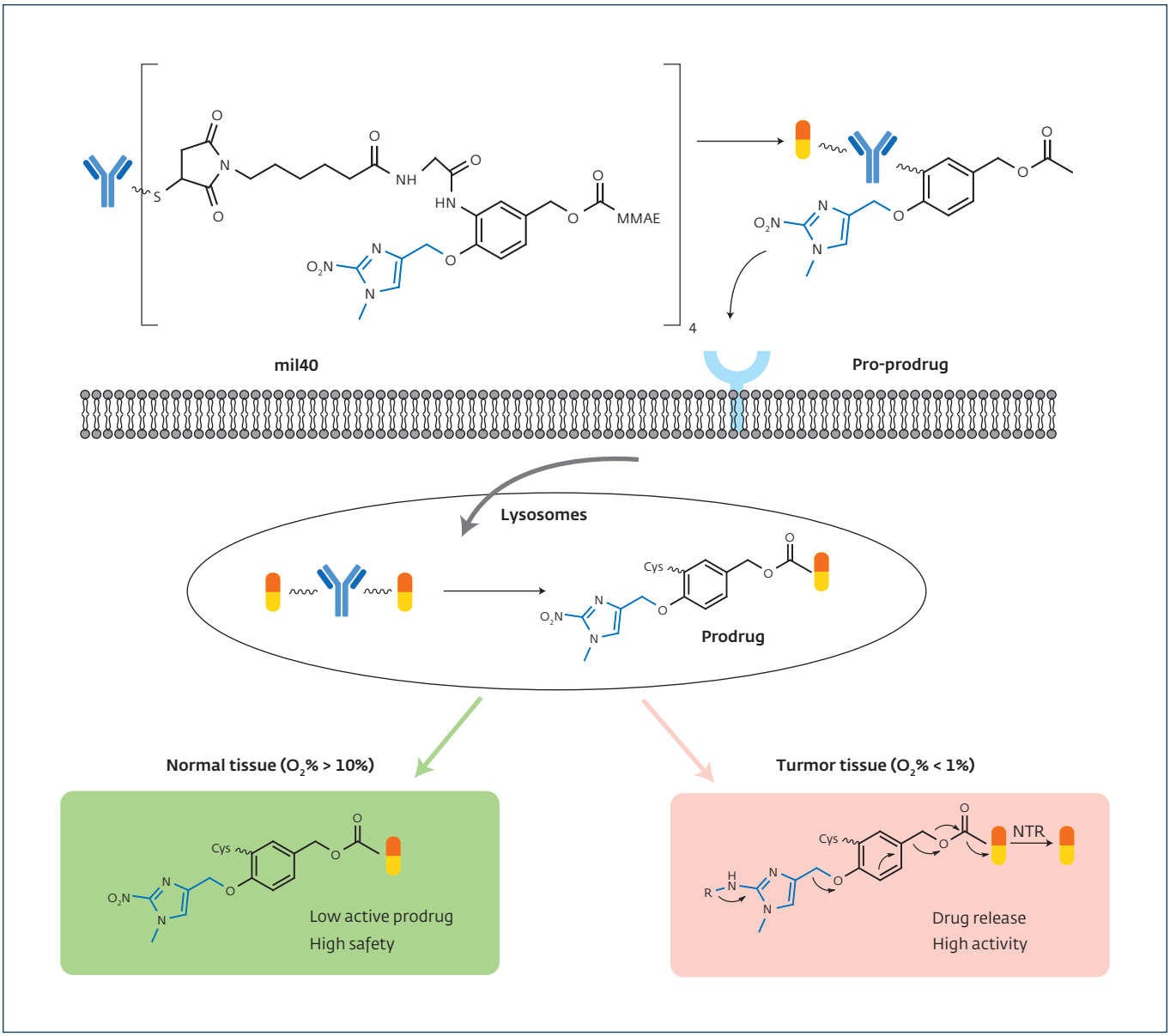

Fig.13. *Structure and mechanism of action of an ADC conjugate with a hypoxia-responsive linker*

cer. Thus, the level of total hydrogen peroxide, the most stable representative of ROS, in healthy tissues lies in the range of 0.5–7 μM, while in a tumor its concentration can increase up to 1 mM [28]. This feature of cancer cells makes it possible to develop linkers that release the active substance in response to increased ROS levels.

For example, the authors of [29] proposed using a linker containing a combination of phenylboronic acid and a carbamate moiety. In the presence of hydrogen peroxide, the C-B bond is oxidized to form arylboronate, which in turn undergoes rapid hydrolysis to p-phenol. As a result of the subsequent transformations, the cytotoxic substance attached to the carbamate group is released. The authors synthesized MMAE conjugates with trastuzumab and durvalumab and demonstrated that the carbamate linker including arylboronic acid remains stable in human plasma, but readily releases the active component in the presence of hydrogen peroxide both inside the cancer cell and in the extracellular space in the tumor (Fig.12).

Hypoxia-responsive linkers

One of the most characteristic features of solid tumors is hypoxia, the level of which correlates with a poor prognosis among cancer patients. Hypoxia develops as a result of uncontrolled cell proliferation, impaired metabolism and damage to the structure of blood vessels, which together leads to slow

transport of oxygen and building blocks. While hypoxia is lethal to healthy cells, tumor cells not only perfectly adapt themselves to low oxygen levels, but also become resistant to chemotherapy and radiotherapy [30].

The oxygen level in tumor cells is 1%, while in normal cells it is more than 10%, and this difference leads to a 100-1000-fold increase in nitroreductase levels.

Hypoxia-sensitive carbamate linkers contain para-nitrobenzyl or nitroimidazole moieties. In a hypoxic cancer environment, nitroreductase reduces the nitro group to an amine, and the subsequent redistribution of electron density leads to the release of the active component.

In xenograft mouse models, the effectiveness of ADCs containing the active component MMAE connected to anti-HER2 antibodies (mil40) through p-nitrobenzyl- and nitroimidazole-based linkers were demonstrated [31]. At the same time, 100% stability of the nitroimidazole linker in plasma was demonstrated, providing a high maximum tolerated dose (MTD, LD10) (Fig.13).

* * * *

The correct choice of linker plays a critical role in the development of chimeric molecules targeting tumors. An ideal linker should be stable in the circulatory system until it enters the tumor or be excreted unchanged from the body. Any premature release of the active component results in unwanted toxicity. Upon penetration into cancer tissue under the influence of specific stimuli, such as increased acidity, reductive medium, high levels of ROS with low levels of molecular oxygen, as well as the expression of certain enzymes, the linker is cleaved and the active component is released. The development of effective and safe linkers remains an extremely pressing task.

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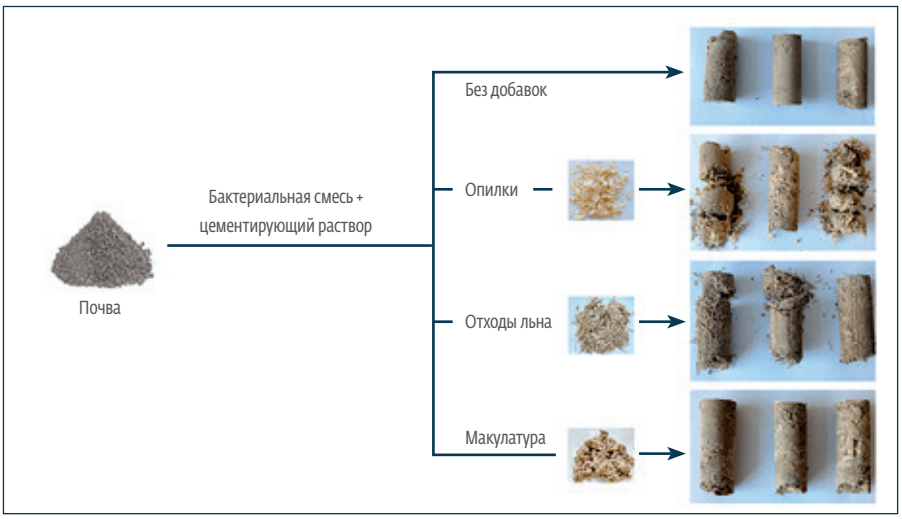
Технология стабилизации почвы с помощью бактерий



Грунт с заданными геотехническими свойствами является обязательным условием на начальном этапе любого инфраструктурного строительства. Традиционно для укрепления грунта применяют химические реагенты, таких как бентонит, силикаты или акриламид. Однако в последнее десятилетие активно разрабатывается технология биоцементации с использованием специальных уреолитических бактерий, осаждающих кристаллы карбоната кальция. Один из таких методов – технология микробно-индуцированного осаждения кальцита (МИКП). В ней используются широко распространенные в природе штаммы бактерий, способные вызывать осаждение карбоната кальция за счет своей метаболической активности. Механизмы микробной индукции минерализации основаны на многочисленных ферментативных реакциях, приводящих к образованию биоцемента из карбоната. Однако высокая стоимость компонентов ограничивают широкое применение МИКП.

Одним из возможных решений является включение органических отходов в почвы. Группа ученых НИЦ "Курчатовский институт" – Петербургский институт ядерной физики им. Б.П. Константинова предложили заменить дорогостоящие реагенты для производства бактериальных инокулятов отработанными пивными дрожжами (ОПД), а также макулатурой, отходами льна и опилок в качестве укрепляющих добавок в почву. Эксперименты оказались успешными, замена труднодоступных компонентов среды LB экстрактом ОПД увеличивает ростовые характеристики биомассы штаммов Bacillus subtilis K51, B. cereus и Micrococcus luteus в 1,4, 1,5 и 1,8 раза соответственно, а для штамма B. subtilis 168 они были сопоставимы со средой LB. Среди трех видов целлюлозосодержащих отходов включение макулатуры в образцы почвы, обработанные МИКП, привело к увеличению прочности на сжатие в 2,1 раза и увеличению процента кальцита почти в 1,5 раза по сравнению с образцом без добавок.

Внесение в почву отходов льна и опилок снижало прочностные характеристики образцов.

Ученые надеются на широкое распространение предложенного метода и подчеркивают, что технология может также использоваться для ремонта трещин в цементобетонных конструкциях, для предотвращения оседания песчаного грунта и разрушения склонов.

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